Sensitive Detection of Trichloroanisole (TCA) in Wine Using Triple Quadrupole GC/MS

Application Note

Foods and Flavors

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Abstract

A method for the detection and quantification of 2,4,6-Trichloroanisole in wine at a concentration as low as 1 ppt was developed using the Agilent 7890A/7000 Series Triple Quadrupole GC/MS with a Pressure Controlled Tee configuration.

Introduction

Haloanisoles are known to cause undesirable flavors in food and beverages, with one of the most notable examples being "cork taint". Cork taint imparts a moldy, musty, or "wet newspaper" flavor to wine and is due to the presence of a range of aromatic compounds, with the major contributor being 2,4,6-trichoroanisole (TCA). The olfactory threshold of 2,4,6-TCA ranges from 1.4-4.6 ng/L, also expressed as parts per trillion (ppt), depending on the panel, methodology and wine matrix [1]. The production of TCA in wine is complex, but is often associated with the conversion of chlorophenols, generated during cork bleaching or by industrial pollution, to TCA by airborne fungi.

Detection of TCA in wine is accomplished by headspace solid-phase-microextraction (HS-SPME) GC/MS analysis, and an HS-SPME High Resolution Mass Spectrometry (HRMS) method in the electron ionization (EI) mode has recently been reported with a limit of detection (LOD) of 0.67 ng/L (ppt) in white wine [2]. This application brief describes a method using the Agilent 7000 Series Triple Quadrupole GC/MS which generates sensitivity of detection comparable to the HS-SPME HRMS method, without the significant financial investment and highly trained operators required to assure valid results with HRMS. The method utilizes EI and GC backflushing to enable detection of 2,4,6-TCA at levels as low as 1 ppt, with a signal to noise ratio of 30:1, indicating a very low LOD.

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Experimental

Standards and Reagents

The standards and reagents used are listed in Table 1. The final solutions of 2,3,6- and 2,4,6-trichloroanisole were made in 13% Cabernet Sauvignon. 2,3,6-TCA was used as an internal standard.

Table 1. Standards and Reagents

Standard	2,3,6-Trichloroanisole	Sigma-Aldrich	99.9% purity
	2,4,6-Trichloroanisole	Sigma-Aldrich	99.8% purity
Reagents	Sodium Chloride	Sigma-Aldrich	99% purity
	Cabernet Sauvignon	Vendange Winery	13% alcohol by vol.

Instrument

The experiment was performed on an Agilent 7890A Gas Chromatograph equipped with a split/splitless capillary inlet and an Agilent 7000 Series Triple Quadrupole GC/MS with Triple-Axis Detector. The split/splitless inlet is fitted with a long-lifetime septum (p/n 5183-4761) and a deactivated, splitless single taper injection liner (p/n 5181-3316). HS-SPME injections were made using a manual SPME Holder (Supelco, 57330-U). The instrument conditions are listed in Table 2.

Table 2. Gas Chromatograph and Mass Spectrometer Conditions

GC Run Conditions	
Analytical column	Two 15 m × 0.25 mm, 0.25 µm Agilent J&W HP-5ms SUI columns (p/n 190915-433UI)
Injection	SPME; 2 min; 250 °C; 50 mL/min purge at 2 min
Carrier gas	Helium, Constant Flow, 3 mL/min
Oven program	40 °C (2 min hold), 25 °C/min to 215 °C
Transfer line temp	280 °C
GC Post-Run Conditions	
Backflush device	Purged Ultimate Union (p/n G3186-60580) controlled by a Pressure Control Module (p/n G3476-60501)
Backflush conditions	-5 mL/min at 250 °C for 2 min
MS conditions	
Tune	Autotune
EMV Gain	20
Acquisition parameters	EI; selected reaction monitoring
Collision gas flows	Nitrogen at 1.5 mL/min, Helium at 2.35 mL/min
Solvent delay	6.5 minutes
MS temperatures	Source 300 °C; Quadrupoles 150 °C

A 1-meter Agilent J&W HP-5ms Ultra Inert (UI) column was used as a guard, and a backflushing configuration was employed to allow for easy inlet and column maintenance. The guard column was connected to the first analytical column via an ultra low dead volume Ultimate Union. Two 15-m HP-5ms UI analytical columns were connected by a Pressure Controlled Tee configured with a Purged Ultimate Union [3-5]. The guard column protects the analytical column from any contaminants that are adsorbed onto the SPME fiber and allows servicing of the inlet without the potential to oxidize the analytical column.

Sample Preparation

All samples were prepared in 20-mL headspace vials. Samples of 2,4,6-TCA were prepared from the 2,4,6-TCA stock solutions at concentrations of 1, 5, 10, 15, and 20 ng/L, in 13% alcohol (v/v) Cabernet Sauvignon wine containing 10 ng/L 2,3,6-TCA as an internal standard. Two grams of sodium chloride was added to each 10-mL aliquot of sample to increase extraction efficiency. HS-SPME was performed on each aliquot using a conditioned 50/30 µm DVB/Carboxen /PDMS StableFlex SPME fiber, (Supelco p/n 57329-U). Samples were subjected to 30 min of static HP-SPME extraction at room temperature, and the fibers were desorbed for 2 min in the GC inlet at 250 °C.

Analysis Parameters

The parameters used in the analysis of 2,3,6- and 2,4,6-TCA are shown in Table 3.

Table 3. Triple Quadrupole GC/MS Analysis Parameters

Compound	RT (min)	SRM	Dwell Time (ms)	Collision Energy (EV)
2,4,6-Trichloroanisole	7.594	210→195	25	15
		167→83	25	20
2,3,6-Trichloroanisole	7.867	210→195	25	10
		210→167	25	20
		167→83	25	20

Results

Sensitivity Comparable to HRMS

The method developed on the 7000 Triple Quadrupole GC/MS system using EI provides ultralow detection of 2,4,6-trichloroanisole in a complex matrix with minimal interferences (Figure 1). The signal-to-noise ratio (S/N) for 2,4,6-TCA at 1 ng/L was greater than 30:1. The calibration curve determined with 2,4,6-trichloroanisole in wine from 1 to 20 ng/L has an R^2 of 0.993 (Figure 2). The high level of sensitivity achieved, which is very comparable to that achieved with GC/HRMS [2], is due in large part to the selectivity of the 7000 Triple Quadrupole GC/MS using EI.

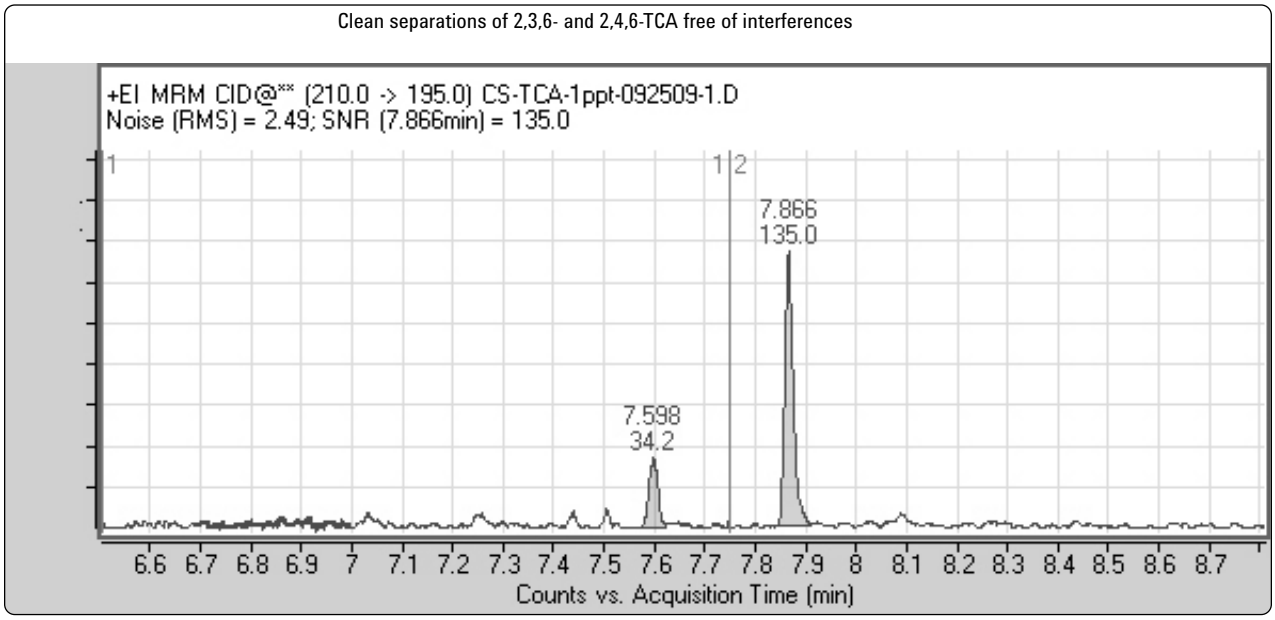

Figure 1. Reconstructed Total Ion Current Chromatogram (RTICC) resulting from SRM analysis, showing the separation of 2,3,6- and 2,4,6-TCA in a sample of Cabernet Sauvignon wine spiked with 10 ng/L 2,3,6-TCA and 1 ng/L 2,4,6-TCA. The 2,4,6-TCA spiked sample elutes at 7.598 minutes, and the 2,3,6-TCA internal standard elutes at 7.866 minutes.

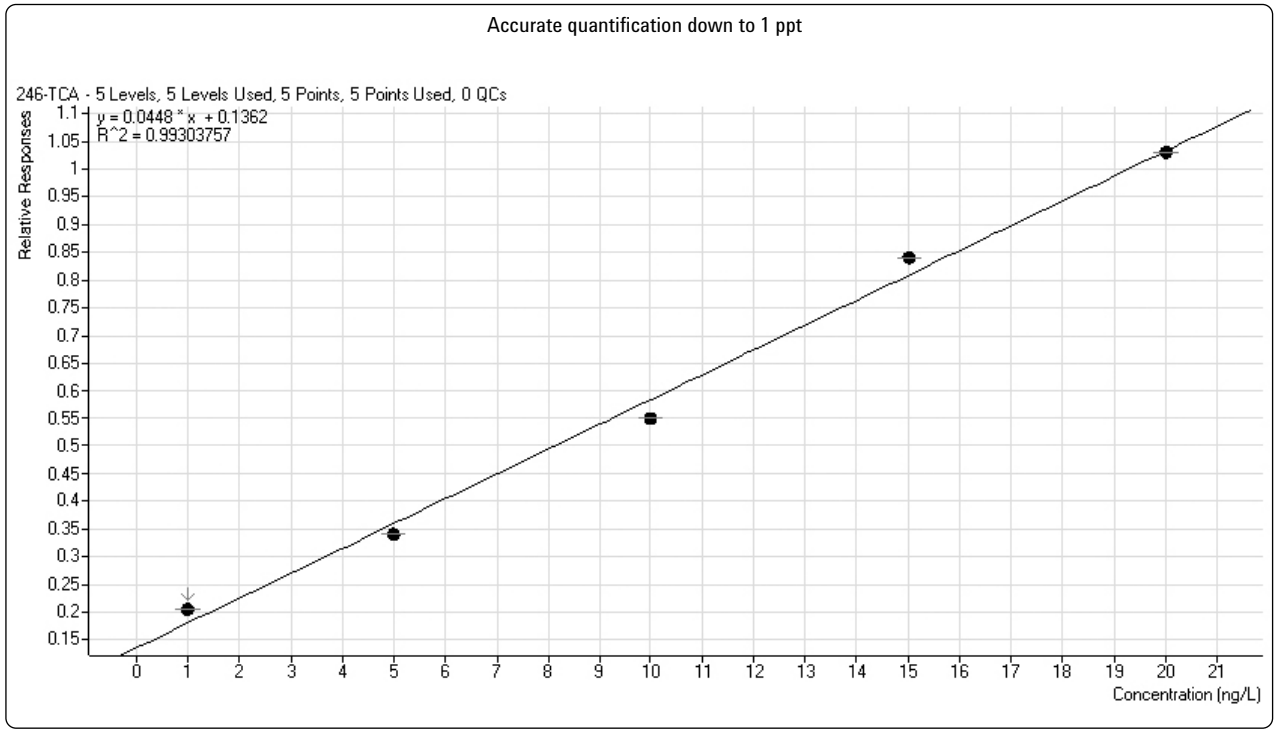

Figure 2. Calibration curve for quantification of 2,4,6-TCA. Samples containing 1, 5, 10, 15 and 20 ng/L of 2,4,6-TCA in Cabernet Sauvignon wine were used to construct the curve.

Conclusion

The use of EI mode with backflushing configuration on the new Agilent 7000 Series Triple Quadrupole GC/MS minimizes interferences and enables the detection of 1 ppt 2,4,6-TCA in wine at an S/N over 30:1.

References

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