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Analysis of 2,4,6-trichloroanisole in wines using solid-phase microextraction coupled to gas chromatography–mass spectrometry

Thomas J. Evans, Christian E. Butzke, Susan E. Ebeler*

Department of Viticulture and Enology, University of California, Davis, CA 95616, USA

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Abstract

Cork taint in wine is a serious problem which is exacerbated by the difficulty of its assessment. Current analytical procedures are costly, time consuming and require the use of large amounts of solvents. We developed and evaluated a rapid method for the detection of the cork taint compound, 2,4,6-trichloroanisole (TCA), in wine samples. The method employs solid-phase microextraction, a solventless, automated sampling procedure, coupled to GC–MS-selected ion monitoring analysis. Quantification is enabled by a fully deuterated [$^2H_5$]TCA analog used as an internal standard. Accuracy (±8%), precision (R.S.D. 5–13%), and limit of quantification (5 ng/l) are comparable to existing methods. © 1997 Elsevier Science B.V.

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1. Introduction

Cork taint, a moldy/musty off-odor in affected bottles, is one of the most serious problems affecting the wine industry. Estimates of global dollar loss range up to US$ 10 billion [1]. Reasonable estimates of the incidence of corked bottles range from 2.5 to 5% [1–3]. 2,4,6-Trichloroanisole (TCA), while not the only causal agent, is certainly the most commonly encountered. One group of researchers [4] observed that TCA was present in 62% (23 out of 37) of the tainted wines they analyzed. An additional report suggests that TCA is present in 70–80% of all tainted wines [1]. In a recent study of Australian wines [5], 100% of the tainted (sensory assessment by wine industry personnel) wines had TCA at or above the sensory threshold value.

TCA is introduced to wines via defective cork closures ([5]; for review see Ref. [6]). The human sensory threshold (reported as the average of a group of individuals) is in the range 1.4–10.0 ng/l (variability due to type of wine used, sensitivity of judge, and training of judge), concentrations which are beyond the sensitivity of most analytical systems without a concentration step. One widely used analytical method, that of Buser et al. [7], employs a pentane–ethyl acetate (3:1) solution as the extracting solvent and a 2500:1 concentration. In spiked additions ranging from 30–100 ng/l, recoveries of 43–72% have been reported with quantification via an external standard procedure. Whitfield [8] employed a similar technique for the analysis of 2,3,4,6-tetrachloroanisole, pentachloroanisole and 2,4,6-TCA in fiberboard packing material and in dried fruit. Using 3,5-dimethyl-2,4,6-trichloroanisole as an internal standard (I.S.) they obtained recoveries of 82–105%

*Corresponding author.

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for the three analytes in the different sample matrices. In fiberboard, standard deviations for peak area ratios of TCA to I.S. in the range of 0.006–3.0 were obtained with relative standard deviations (R.S.D.s) of 5–8%.

Solid-phase microextraction (SPME) offers a quick, accurate, and inexpensive tool for analysing volatile components, including TCA, in various sample matrices. Successful adaptation of the SPME technology to the quantification of TCA in wines offered the incentive of reduced analysis time and reduced cost due to the elimination of extracting solvents. The SPME device, manufactured by Supelco (Bellefonte, PA, USA), consists of a 1 cm piece of fused-silica with an adsorbent material bonded to the *outside*. The fused-silica is attached to the end of a metal fiber and the entire assembly can be retracted inside a hollow needle slightly larger than that of a standard GC syringe. Sampling the headspace is accomplished by exposing the fiber inside the sample vial. Analytes are concentrated on the fiber based on their partition coefficients between the liquid, the gas phase above the sample, and the fiber [9,10]. To perform injections, the assembly functions like a GC needle by puncturing the septum of the injection port; the fiber, once in the injector, is exposed (via the retractable mechanism) and the analytes are thermally desorbed and swept on to the column by the carrier gas. The SPME fiber obviates the solvent removal step typically required with liquid–liquid extraction procedures [7]. Evaporation of the concentrating solvent, which can take several hours, is eliminated, and under automated conditions the length of the sample preparation equals the adsorption time of the fiber for each sample. Our objective was to evaluate the SPME method combined with use of a fully deuterated internal standard ($[^2H_5]$TCA), for quantification of TCA in wine headspace.

2. Methods and materials

2.1. Instrumental analysis

A Varian 8200cx autosampler (Walnut Creek, CA, USA) mounted to a Hewlett-Packard 5890 gas chromatograph (Avondale, PA, USA) paired with a Hewlett-Packard 5971 mass selective detector (with an electronics upgrade to a Model 5972) constituted the analytical system. The software used was MSD ChemStation by Hewlett-Packard.

The gas chromatograph was fitted with a fused-silica capillary column with a dimethylpolysiloxane stationary phase and dimensions: 25 m×0.25 mm I.D, 0.25 μm film thickness (CP-SIL 5 CB; Chrompack, Raritan, NJ, USA). Columns with other phases were also evaluated in preliminary stages of the investigation including, diphenyldimethylpolysiloxane (DB-1; J&W, Folsom, CA, USA), and poly(ethylene glycol) (DB Wax, J&W; and Stabilwax, Restek, Bellefonte, PA, USA). The GC injector was equipped with a straight glass liner, interior diameter 0.7 mm. The oven parameters were as follows: initial temperature 45°C and held for 2 min, then increased to 265°C at a rate of 12°C/min and finally held for 1 min (total cycle time including oven cool-down ≈26 min). Injections were splitless, the purge valve was activated at 3 min, and the injector was held at 260°C. Temperature of the transfer line to the MS system was 280°C. Helium was the carrier gas at 45 cm/s.

For the detector, the electron multiplier was manually adjusted to 1976 eV (an increase of 294 eV over the autotune value). The solvent delay was set at 9 min (to save wear on the filament at this elevated voltage). The dwell was set at 200 ms and two ions were monitored, m/z 195 for TCA and m/z 215 for the deuterated analog.

The autosampler was fitted with a polydimethylsiloxane (PDMS) SPME fiber with a phase thickness of 100 μm. Headspace sampling was done from 16 ml vials each containing a total of 10 ml of liquid sample. The autosampler was programmed for a 28 min cycle; 3 min for desorption in the GC injector and 25 min adsorption time for sampling the headspace. This cycle time was slightly longer than that of the GC analysis time so that the fiber was always in either the injector or the headspace of a vial in order to minimize analyte loss and contamination of the fiber by ambient air.

A hooded electric light assembly fitted with a 25 W flood type bulb was suspended directly over the autosampler at a height of 2–3 cm above the samples in the autosampler tray. Samples were held under the light for 20 min prior to analysis. This apparatus

maintained a constant temperature, 45°C, and enhanced the volatility of the analyte resulting in an increase in the sensitivity of the assay by a factor of 3.

2.2. Reagents and other materials

2,4,6-Trichloroanisole (99%) (Aldrich, Milwaukee, WI, USA), in solid crystal form, was used for calibration. The internal standard, [$^2H_5$]TCA, was generously supplied by the Australian Wine Research Institute (Glen Osmond, Australia) where it was synthesized [5]. Silicone septa from Scientific Resources (Eatontown, NJ, USA) were used with 20 mm crimp caps to seal the sample vials. The septum must provide a good seal for the sample, and must also allow the blunt, slightly oversize bore SPME needle to pierce the septum without damage to the fiber inside.

2.3. Internal standard and sample preparation

A stock solution (2.5 μg/l) of internal standard was prepared in 100% ethanol (Rossville Gold, Hayward, CA, USA). A 1.0 ml aliquot of the 2.5 μg/l internal standard stock solution was added to 9.0 ml of sample. This yielded a final level of 250 ng/l deuterated I.S. in the sample for analysis.

2.4. Statistical analysis

Software for statistical analysis was The EP Evaluator (method comparison, precision, linearity) by David G. Rhodes Associates (Kennett Square, PA, USA).

3. Results

TCA and the deuterated analog are easily recognized by characteristic double peaks in the total ion chromatogram (TIC) at a retention time of ~11 min (Fig. 1, left). At lower concentrations the TCA appears as a shoulder of the deuterated I.S. in the TIC, however, inspection of the extracted ions (Fig. 1, right) reveals two distinct peaks. The m/z 195 $(M-15)^+$ fragment ion of TCA was monitored and it does not occur in the deuterated analog; it is also the most abundant species, which is a factor at trace analyte levels. For the deuterated analog, the molecu-

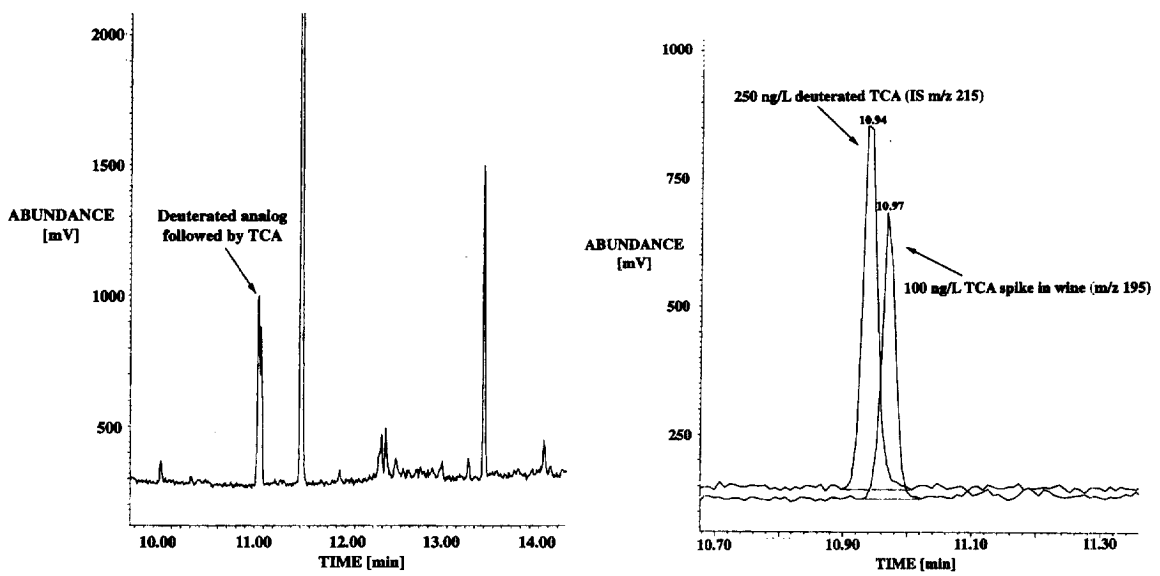

Fig. 1. (a) GC–MS trace (TIC) of 2,4,6-TCA, 100 ng/l, and the deuterated internal standard, 250 ng/l. The I.S. elutes first (t_R = 10.94 min). Identities were determined by GC–MS. (b) GC–MS-selected ion monitoring trace of extracted ions. The TCA fragment at m/z 195 and deuterated I.S. molecular ion (m/z 215) were monitored and used for quantitation.

Table 1
Precision assay for three levels of TCA spiked in a white wine ($n=10$ at each level)

Spiked TCA level (ng/l)	Measured mean value (ng/l)	Range (ng/l)	S.D. (ng/l)	R.S.D. (%)
10	10.88	9.9–11.36	0.551	5.1
25	27.1	21–31	3.5	13.1
250	256.9	220–314	32.6	12.7

lar ion, m/z 215 (M^+) was monitored. This ion does not occur in TCA.

3.1. Linearity and limit of quantification

A calibration curve was generated using TCA. The preparation was identical to that of the internal standard except that after the TCA crystals were solved in 100% ethanol, the subsequent serial dilutions were done in a white varietal box wine, which contained no detectable TCA. Integrated peak area ratios (peak area of TCA/peak area of I.S.) were calculated and plotted against the concentration ratio (ng/l TCA/ng/l I.S. for each standard. The resultant curve was linear [response ratio=(0.531× concentration ratio)+0.0172] with a coefficient of determination (r^2) of 1.000 at the following concentrations: 0, 5, 10, 25, 50 and 250 ng/l (four replicate analyses at each concentration). Quantification was reliable down to 5 ng/l with an estimated signal-to-noise ratio of 8:1.

3.2. Accuracy and precision

Precision assays were conducted at the 10, 25 and 250 ng/l level (Table 1). Measured values were within 3–8% of the theoretical concentration with R.S.D.s ranging from 5–13%. Additional trials were done by adding a 10 ng/l spike to red and white wines with a previously measured quantity of TCA (Table 2). These wines were reported to be tainted by informal sensory analysis and the recovery of spiked TCA averaged 107.8% over a wide concentration range.

4. Discussion

A non-polar column (25 m×0.25 mm, 0.25 μm, CP-SIL 5 CB, Chrompack) was selected for its very low bleed which enhanced sensitivity. Polar poly(ethylene glycol) columns (DB Wax and Stabilwax) were also evaluated. Their polarity allowed shorter analysis times; however, the time required for adsorption of analytes on the SPME fiber exceeded the retention time of the analytes in the nonpolar columns. The magnitude of noise in the baseline of the polar columns was greater than for the non-polar columns, and became limiting at low TCA levels.

SPME splitless injections of samples with trace levels of analytes usually require a straight narrow injector liner (e.g., 0.7–1.0 mm) to minimize peak widths of early eluting peaks (<5 min). In previous studies, a negative effect on early eluting compounds was observed with a 3.5 mm wide diameter liner for

Table 2
Recovery trial for TCA spiked into 'tainted' wines

Wine sample	Initial measured concentration (ng/l)	Measured conc. after 10 ng/l spike (ng/l)	Recovery of spike (%)
1994 Chardonnay	0.0	13.0	130
1994 Pinot noir	4.0	14.3	103
1992 Pinot noir	26.5	35.0	85
1994 Pinot noir	3.2	11.9	87
1994 Zinfandel	119.4	131.4	120
1995 Zinfandel	4.1	16.3	122

The TCA level of wines were quantified using the SPME procedure, a 10 ng/l spike of TCA was added, and the wines were reevaluated using the SPME method.

the analysis of fruit juice volatiles [11]. For TCA, which elutes at ~11 min, no difference in peak width was observed, however, with a 4.0 mm I.D. liner that was constricted at the exit end and contained no glass wool. There is an advantage to the larger diameter liner because the SPME apparatus, with its oversize syringe tends to core septa. In the smaller liner, these bits of septa actually pose a physical barrier which is not the case in the larger liner.

The injector temperature was placed as close as possible to the limit (280°C) of the fiber to ensure swift and efficient desorption. Optimal results were obtained with a moderate oven temperature ramp (12°C/min); if the ramp is too steep excessive background noise due to bleed can compromise sensitivity. The final oven temperature exceeded the injector temperature to eliminate the possibility of carryover.

Due to the volatility of TCA, a headspace sampling method is preferred, reducing the possibility of fiber contamination from the adsorption of non-volatile matrix components. Regarding SPME fiber selection, the PDMS fiber performed better than a polyacrylate fiber due to the relative non-polarity of this particular analyte. Early in the method development, a comparison between two fibers showed that at low mg/l TCA levels, the PDMS fiber adsorbed ~2× more analyte than the polyacrylate fiber.

In an effort to increase the sensitivity of the analysis, NaCl was added to the sample in order to shift the equilibrium toward the headspace. No improvement in sensitivity (measured as peak area) was observed with 1 g additions of NaCl to the sample vial. This procedure, 'salting-out', has been previously reported to enhance the detection of trichlorophenol in water samples [12].

Holding samples at temperatures above room temperature during analysis was critical for optimal sensitivity of the TCA analysis. Use of a constant temperature water bath as compared to the heat lamp has been shown in preliminary trials to enhance precision by a factor of ~2. This indicates that a precisely temperature controlled headspace sampling unit may offer further improvements in the accuracy and precision of the TCA analysis.

The precision and sensitivity of the SPME method are comparable to existing methods. In a recent study, Sefton et al. [5] analyzed TCA in wine samples by extraction with *n*-pentane followed by fractional distillation and concentration in a stream of N_2. At spiked TCA levels of 2 ng/l, they reported a mean value of 2.2±0.4 ng/l over six replicates (R.S.D.=18.2%). While this R.S.D. is higher than that of the SPME method, this trial by the Sefton group is at a slightly lower TCA level than the limit of quantitation (LOQ) of 5 ng/l used in the current study. The liquid–liquid extraction procedure of Buser et al. [7] and Whitfield [8] yielded reported LOQs for TCA of 2–5 ng/l with R.S.D.s of 5–8%. These results are very similar to the accuracy (±8%) and precision (R.S.D.<13%) we observed in this study using the SPME procedure.

5. Conclusions

The combined SPME–GC–MS method described in this paper for the determination of TCA in wine matrices is selective and specific with an LOQ of 5 ng/l. The method allows a rapid, sensitive and precise analysis of TCA in wines, and has the potential to be of further use in the examination of the incidence and causes of cork taint. Preliminary work has been done to expand the application to the simultaneous investigation of other cork-related compounds such as guaiacol (smoky character), geosmin (mildew), 1-octen-3-ol, 1-octen-3-one (mushroom) and 2-methylisoborneol (earthy). These compounds have been implicated as potential sources of odors that can contribute to cork taint [4].

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