Detecting 2,4,6 TCA in Corks and Wine Using the zNose™

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***Abstract* –** For the first time an electronic nose can detect 2,4,6-trichloroanisole (TCA) in wine aromas. Called a zNose™, it is capable of detecting part-per-trillion levels of TCA in less then 10 seconds. Of all the chemical devices under development, an electronic nose to mimic (improve) the human olfactory response while also providing quantitative chemical analysis has been the most sought after by wine producers. Because of its speed and sensitivity, this new electronic nose offers a much needed quality control tool, which can be used to test for quality in virtually every aspect of wine making. The new zNose™ technology is based not upon sensor arrays (eNoses) but instead is based upon a very fast (10 second) gas chromatograph (GC) coupled with a single surface acoustic wave (SAW) solid state vapor detector.

Introduction

This is a need for an electronic nose to detect 2,4,6 TCA directly in wine aroma and corks just like humans do. TCA in wine results in what is commonly called 'corked' wine. The precursors to TCA originate in the wood of the cork tree, Figure 1, and TCA is believed to form when these hydrocarbons combine with chlorine used to bleach the corks before use.

Figure 1- TCA is often introduced into wine from the cork used to seal the wine bottle.

The chloroanisole family of compounds, illustrated in Figure 2, consists of the anisole backbone with increasing numbers of chlorine atoms attached.

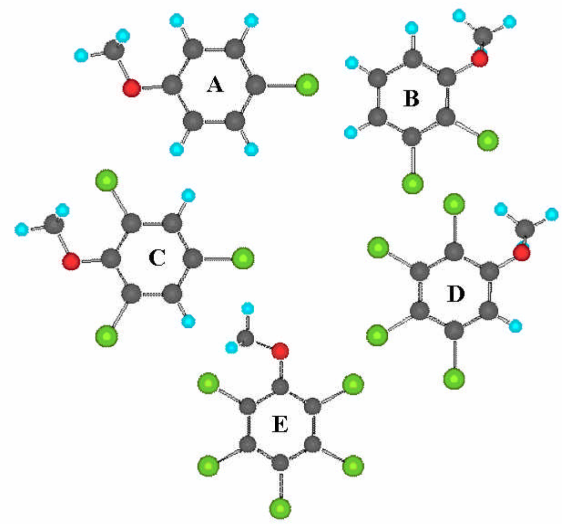


Figure 2- Chloroanisole family consisting of (A) chloroanisole (B) dichloroanisole (C) trichloroanisole (D) tetrachloroanisole, and (E) pentachloroanisole.

One of the main reasons for corkiness in wines has been determined to be 2,4,6-Trichloroanisole. 2,4,6 TCA smells like musty old newspapers. Detailed information on 2,4,6 trichloroanisole is tabulated in Figure 3.

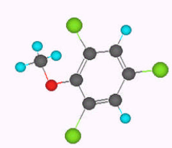
Structure	Formula	Mol. Wt.	NCI No.	CAS No.
	$C_7H_5Cl_3O$	211.48	0035142	87-40-1

Figure 3- Important chemical properties of 2,4,6 Trichloroanisole.

This compound and other halogenated anisols and phenols result from microbiological processes and are formed by dehalogenation and biomethylation. The odor threshold of these substances is in the low ppt range and so their analysis in tainted wines usually requires more or less cumbersome sample preparation like liquid/liquid extraction, solid phase extraction or distillation techniques, often with the drawback of organic solvent use. Measuring ppt levels of TCA in wine is also difficult because there simultaneously is an abundance of other compounds in wine with part per million concentration levels.

The zNose™ – A Fast GC/SAW

The zNose™ is based upon an old technology, gas chromatography. The zNose™, shown in Figure 4, is a small and portable hand-held instrument. Conventional gas chromatographs are much larger instruments, which utilize an ovenized long (10-100 meter) capillary tube (column), coated with a stationary phase to separate or speciate a sample vapor (mobile phase) into its individual chemical components.

A long-column GC is capable of very high resolving power but is also very slow with typical measurements taking 30 minutes to an hour. The zNose™ is a portable GC, which achieves 10-second speed by use of a direct heated, 1-meter column and a new solid state integrating GC detector. Sensitivity for the zNose™ is comparable to a long column GC equipped with an electron-capture detector. High speed (in seconds) requires a trade-off in resolving power, however, sufficient resolution is maintained for many quality control applications such as detecting TCA in wine and drinking water. The zNose™ contains a rechargeable internal helium tank sufficient to perform more than 400 measurements and a vapor preconcentrator so that it is possible to sample weak vapors and preconcentrate them prior to analysis in the fast GC column.

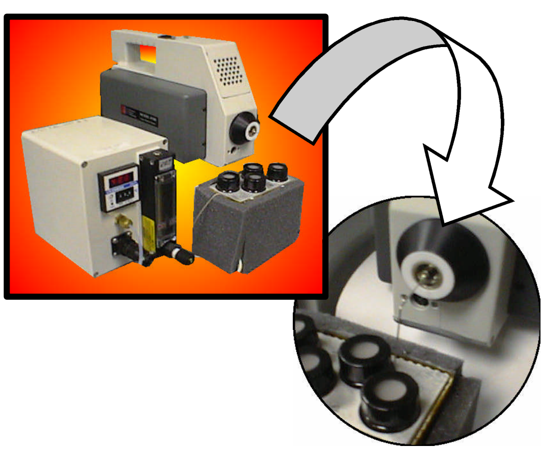


Figure 4- Handheld version of the zNose together with temperature controlled sample holder for 40 mL vials with sealed septa lids.

2,4,6 TCA Calibration

The zNose™ is currently the only electronic nose which can be directly calibrated with chemical standards of known concentration according to recommended EPA/FDA methodology. The response of the zNose™ to headspace vapors from a water source containing 1 part-per-billion (1000 ppt) is shown in Figure 5.

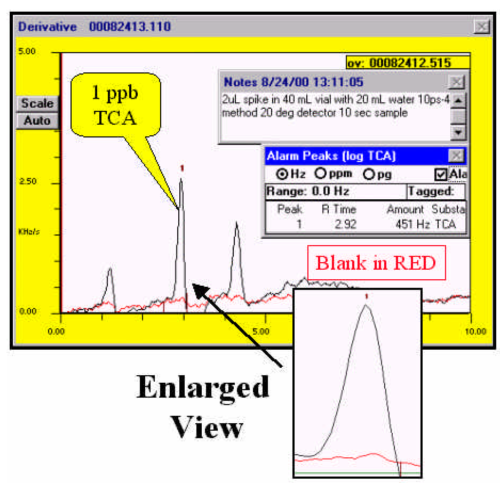


Figure 5- Calibration results using 1 ppb water standard.

Calibration for TCA detection is performed using headspace vapors from water standards. Carefully weighing TCA crystals and dissolving them in methanol produces a spiking solution of known concentration. The water is then spiked with microliter quantities of the methanol spiking solution. The two compounds shown on either side of the TCA response (peak) in Figure 5 are due to trace amounts of dichloroanisole and pentachloroanisole. The water standard consisted of 20 mL water in a septa sealed 40 mL vial. The response of figure 5 was obtained by preconcentrating 5 mL of headspace vapor in 10 seconds followed by a 10 second GC analysis. Under these conditions the signal to noise was 40 to 1 which resulted in a minimum detection level for 2,4,6 TCA in water of approximately 25 parts-per-trillion. Longer sample times (e.g. 60 seconds) routinely allow the zNose™ to achieve detection limits below 1 parts-per-trillion in water.

The zNose™ can also create a virtual sensor specific to TCA. The sensor once calibrated can measure the concentration of TCA vapors contained within a volume of sampled air. This feature is useful when screening wine cellars, storage areas, containers, or cooperage for traces of TCA contamination prior to use. Injecting a microliter of the methanol spiking solution into an air filled 40 mL vial and sampling produces the calibration response as shown in Figure 6. In this case 5 mL of air containing 500 picograms/milliliter TCA produces a response factor of approximately 3.6 Hz/pg. With a noise floor of 10 Hz, the minimum detection level is approximately 3 picograms.

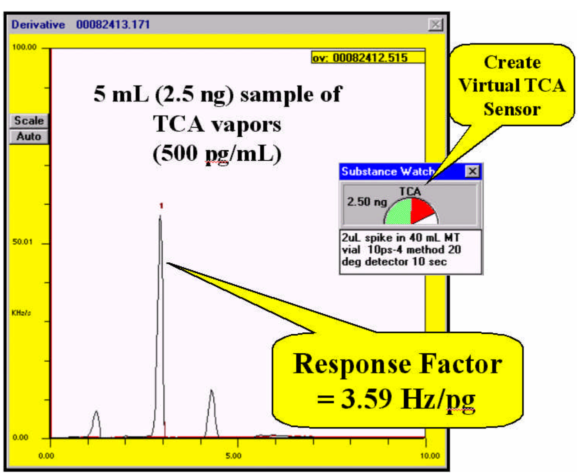


Figure 6- Calibration response obtained by preconcentrating 5 mL volume containing 500 pg of 2,4,6 TCA per milliliter. Virtual sensors can then be created to monitor the concentration of this specific compound

Measurement stability and repeatability, known problems for conventional eNoses, are a thing of the past with zNose™ technology due to the elimination of all chemical coatings, polymers, or other materials, which are known to be unstable over time. Instead the zNose™ uses a single vapor sensor made from single crystal quartz without any absorbent coatings. Under most conditions the zNose™ maintains calibration without adjustment for periods up to 30 days.

Repeatability and precision are demonstrated in the offset-waterfall display of Figure 7. Here consecutive measurements on a sample bottle containing TCA vapors are shown. The systematic reduction in signal level with each measurement is due to the removal of 5 milliliters of vapor with each measurement. Adjusting for this systematic withdrawal, the standard deviation between measurements is typically 2-3%.

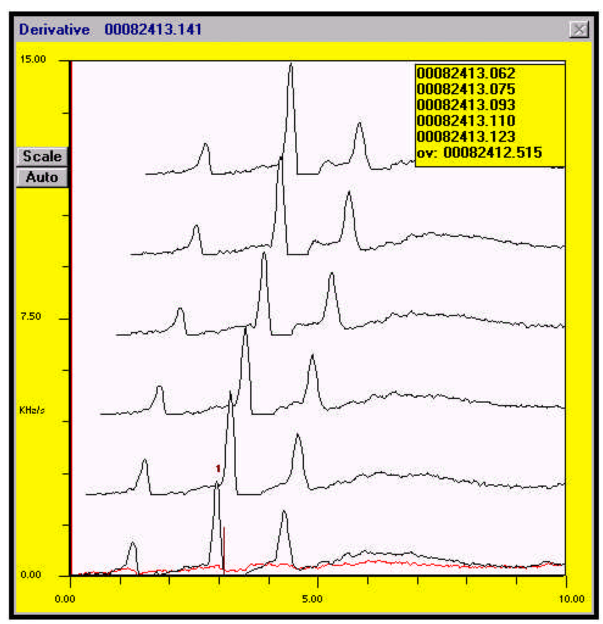


Figure 7- Offset waterfall display showing repeatability of 6 consecutive measurements of a 40 milliliter vial containing DCA, TCA, and PCA vapors. Decrease in signal level is due to removal of 5-milliliter volume with each sample and test.

Detection of TCA in Wine

Measuring part-per-trillion levels of TCA in wine is more difficult than water because there simultaneously can be other compounds in wine at part-per-million concentration levels. This requires an instrument with a very high dynamic range and good baseline separation so that both high and low concentration levels can be accommodated in a single measurement.

A QC test for corks requires soaking the corks in a neutral white wine solution for 24 hours and then testing for the presence of TCA. In most wineries a panel of experts performs the test with an olfactory detection threshold of 5-10 ppt.

A series of 10 neutral wine solutions, identified by an expert panel as having TCA were obtained from a California winery. The aroma from each sample was tested to see if TCA in the aroma could be detected by the zNose™. A representative test result for a neutral wine sample is shown in Figure 8. In each wine sample the

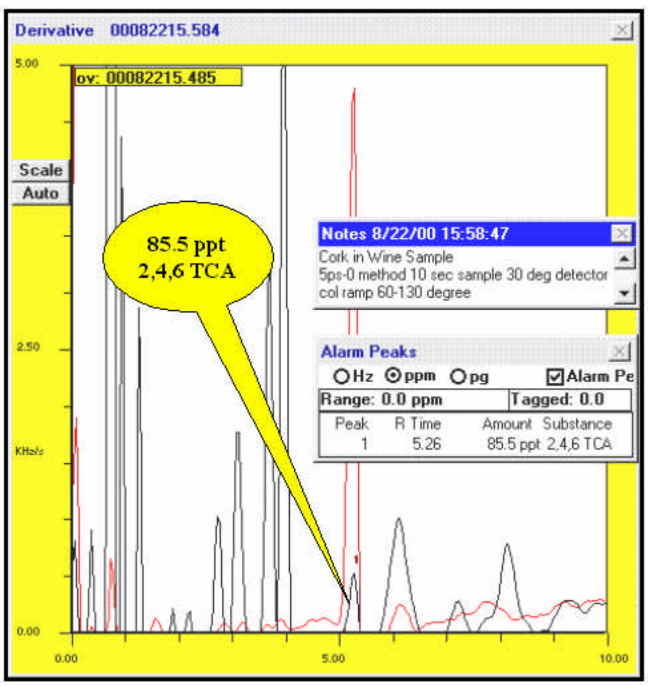


Figure 8- Test chromatogram of a simple wine used to extract TCA from corks. TCA calibration response is shown as overlay in red.

zNose™ was able to detect and quantify the amount of TCA in each wine's aroma. Overlaid in red is the response to a 1 ppb TCA calibration standard. The presence of 85.5 ppt TCA can clearly be seen and as expected was above the odor threshold (5-10 ppt) of the expert panel. In these tests a relatively small 5 millimeter sample of headspace vapor was preconcentrated (10 seconds) with a 10 second GC analysis and a modest 30^{o}C detector temperature. Decreasing the detector temperature and preconcentrating a larger sample of headspace vapor allows the zNose™ to quantify concentrations of TCA below 1 part-per-trillion.

The use of a neutral white wine to extract TCA from corks is widely used in the US however many cork manufacturers use a methanol/water solution. In this case there is less likelihood of encountering an interfering compound with the same retention time of TCA. Nevertheless, in real wines like a Merlot or Chardonnay there are a considerable number of hydrocarbons like caprylate and caproate, which can produce elevated baselines and otherwise mask the presence of TCA if their concentration is above 1 ppm.

Quality in Wine Production

The zNose™ is the first electronic nose to achieve the same or better sensitivity to 2,4,6 TCA as a panel of wine experts. In addition to TCA, there are hundreds of other hydrocarbons in wine and wine production which can either enhance or destroy the quality of the wine. In fact the entire wine process is dominated by hydrocarbons. zNose™ technology currently is able to analyze and quantify all hydrocarbons in wines with molecular weights between approximately 50-300.

The zNose™ is a fast and accurate olfactory tool designed to support and not replace testing by sensory experts. Instruments can never replace the human sensor, however, the ability to quantify and understand the human perception of a wine's chemistry may provide valuable insights into how to define and quantify wine quality in chemical terms. Once the optimum chemistry is defined, instruments like the zNose™ will be able to compare and monitor the chemical composition and quality of the entire wine production process.

Because this paper focus upon taints in wine it should be noted that not all TCA contamination comes from corks and that its presence can also result from persistent microbiological activity within the winery itself. An electronic nose can be used to detect and screen any vessel, container, or building for TCA. It is not uncommon to find TCA in cooperage and wine cellars. Applied as an ambient air monitor, the portable zNose™ is able to detect TCA and many other tainting vapors at sub-ppt levels.

Not all TCA contamination comes from microbial activity. Shipping containers can be a source of chlroanisoles because these compounds can migrate quickly and readily equilibrate and coat all surfaces within the container. Other sources of TCA can be PCP-treated wood used in construction of wine cellars. This can quickly contaminate the atmosphere, wines, barrels, tanks, corks and everything else within the cellar.

Glass bottles must be regarded as another possible source of chlroanisoles and other tainting compounds in bottled wine. The electronic nose is an effective tool for inspecting and quantifying cleanliness in bottles and many other containers used in the production and storage of wines.

An electronic nose can monitor the concentration of composition of fragrances supportive to the overall good taste and bouquet of the wine as well. The electronic nose is a useful tool for maintaining the exact chemical composition of an award winning wine and warning of harmful taints or changes in composition, which could detract from the overall quality of the wine.

Effectiveness

The accuracy and precision of wine testing frequently requires complex testing methodology to be used. Long-column GCs are expensive, not portable, and can only measure 10 samples in an 8-hour day. With zNose technology, more than 320 samples can now be measured in a single day. This is a 30X increase in efficiency and results in a very short pay-back period for the instrument.

TCA in delivered wines is relatively rare, occurring in only 1-2% of wines shipped. This is a result of effective testing and screening by wineries before their product is shipped. Actual amounts of wine rejected (destroyed) due to TCA tainting are not as well known, however, the number is believed to be significantly higher. These losses represent a significant potential gain in terms of more wine shipped and customer satisfaction and are cost effective reasons to employ electronic noses in wine production.