AN ABSTRACT OF THE THESIS OF

Peter M. Davis for the degree of Master of Science in Food Science and Technology presented on March 30, 2012

Title: The Effect of Wine Matrix on the Analysis of Volatile Sulfur Compounds by Solid-Phase Microextraction-GC-PFPD

Abstract approved:

Michael C. Qian

Constituents of the wine matrix, including ethanol, affect adsorption of sulfur volatiles on solid-phase microextraction (SPME) fibers, which can impact sensitivity and accuracy of volatile sulfur analysis in wine. Several common wine sulfur volatiles, including hydrogen sulfide (H_2S), methanethiol (MeSH), dimethyl sulfide (DMS), dimethyl disulfide (DMDS), dimethyl trisulfide (DMTS), diethyl disulfide (DEDS), methyl thioacetate (MeSOAc), and ethyl thioacetate (EtSOAc), have been analyzed with multiple internal standards using SPME-GC equipped with pulsed-flame photometric detection (PFPD) at various concentrations of ethanol, volatile-, and non-volatile-matrix components in synthetic wine samples. All compounds exhibit a stark decrease in detectability with the addition of ethanol, especially between 0.0 and 0.5%v/v, but the ratio of standard to internal standard was more stable when alcohol concentration was greater than 1%. Addition of volatile matrix components yields a similar decrease but the standard-to-internal-standard ratio

was consistent, suggesting the volatile matrix did not affect the quantification of volatile sulfur compounds in wine. Non-volatile wine matrix appears to have negligible effect on sensitivity. Based on analyte:internal standard ratios, DMS can be accurately measured against ethyl methyl sulfide (EMS), the thioacetates and DMDS with diethyl sulfide (DES), and H_2S, MeSH, DEDS, and DMTS with diisopropyl disulfide (DIDS) in wine with proper dilution. The developed method was then used to quantify sulfur compounds in 21 various California wines. H_2S and MeSH were found in higher concentrations in white varietals, while DMS was slightly higher in red varietals, particularly cabernet sauvignon and merlot. Trace amounts of DEDS and MeSOAc were found in almost all wines. DMS and DMTS were found in all wines, in some instances above reported thresholds.

The Effect of Wine Matrix on the Analysis of Volatile Sulfur Compounds by Solid-Phase Microextraction-GC-PFPD

by

Peter M. Davis

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Peter M. Davis, Author

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INTRODUCTION: PROGRESS ON VOLATILE-SULFUR-COMPOUND ANALYSIS IN WINE

Sulfur

Sulfur is an abundant and naturally occurring element. Sulfur has the atomic number 16 with four natural isotopes, primarily ^{32}S (approx. 95%) and ^{34}S (approx. 4%), that average to an atomic weight of 32.065(5)amu. Set directly beneath oxygen in the periodic table, sulfur is the second member of the group 16 family of elements known as the chalcogens. In its natural, elemental solid state, sulfur is composed of eight-membered rings stacked upon each other in an ordered fashion, exhibiting a dull yellow color. It often accumulates around volcanic openings, and was known by the ancients as *brimstone*. Polysulfides, involving chains of sulfur-sulfur bonds, are not uncommon, though elemental sulfur defaults most naturally to cyclic S_8 [1, 2].

Sulfur's self-affinity has enormous biological and technological significance. Introduction of the vulcanization of rubber in the 19th century revolutionized industrial machines, relying on the cross-linking of natural rubber polymers with polysulfide bonds to improve cohesion and restriction. Disulfide bonds formed between cysteine residues in biological macromolecules have importance in protein stability and irreversible substrate binding (also a notable device in medicinal chemistry), trumping more prevalent hydrogen bonds and intermolecular forces with its covalent strength.

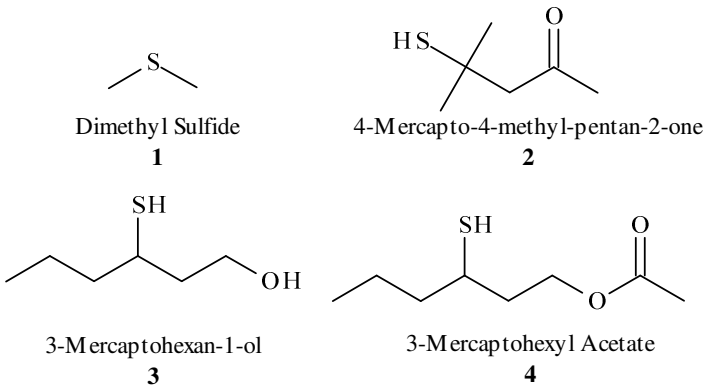
Numerous examples of disulfide bonds in biological systems abound, reinforcing the great importance of sulfur in biology, living organisms, and food systems. However, the tendency to form disulfide bonds creates difficulty for chemical analysis, disturbing the natural analyte system during testing.

Volatile sulfur compounds (VSCs)

Volatile sulfur compounds (VSCs), especially at lower molecular weights, are known for giving off strong, offensive odors. Hydrogen sulfide is likely the most well-known VSC, characteristic of rotten eggs. Cabbage patches are a familiar reference for sulfurous odors, as the existence of smaller thiols and sulfides in many cultivars of cabbage is well known [3]. Other noted sources of VSCs are allium vegetables such as onion, garlic, and chive [4-7], asparagus [8], broccoli and cauliflower [9], tropical fruits like grapefruit, guava, and passion fruit [10-13], lychee [14], *etc.* in which VSCs occur naturally, and roasted systems having undergone Maillard reaction such as cooked meats [15-17], toasted sesame seeds [18], and coffee [19-21]. Many sulfur volatiles have extremely low odor thresholds, many in the parts per trillion (ppt) range [22], contributing significantly to overall aromas. In many cases, this is a less-than-desirable effect. Commonly accepted theory of perception suggests that highly volatile small sulfur compounds elicit a strongly negative response to warn consumers of rotten or spoiled foods; many odor-active products are formed through decomposition and putrefaction [23, 24].

However, not all VSCs are necessarily foul-smelling. It has been suggested that very minute amounts of certain sulfur compounds, including the usual low-molecular-weight offenders, can actually enhance and benefit the

aroma of certain foods, including wines. Dimethyl sulfide (**1**), for instance, has been shown to bring out fruity character in red wines at low concentrations [25, 26]. Some larger structures exhibit some earthy, green, and tropical notes which are of great importance to some varietal character, particularly in Sauvignon blanc [27]. Generally aromas compared to gooseberry, boxtree, black currant, grapefruit, and other tropical fruits are elicited from such compounds, the three most prominent of which are 4-mercapto-4-methylpentan-2-one (**2**), 3-mercaptohexan-1-ol (**3**), and 3-mercaptohexyl acetate (**4**).



Dimethyl Sulfide
1

4-Mercapto-4-methyl-pentan-2-one
2

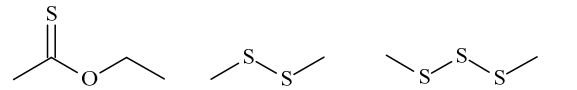
3-Mercaptohexan-1-ol
3

3-Mercaptohexyl Acetate
4

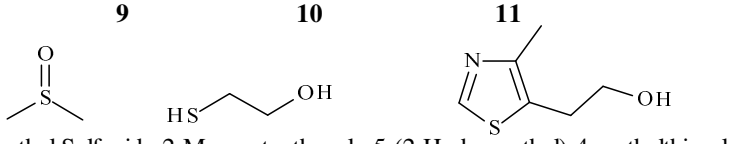
Some of the most noxious sulfur volatiles are small, low-molecular-weight compounds referred to as "light" VSCs. The 'light' indication is not solely based on molecular weight, but on boiling point, which, by definition, falls below 90ºC. These commonly include hydrogen sulfide (H_2S), dimethyl sulfide (**1**), ethyl methyl sulfide (**5**), methanethiol (CH_3SH), ethanethiol (C_2H_5SH), carbon disulfide (**6**), and carbonyl sulfide (**7**). Methyl thioacetate (**8**) is also of note, as, though technically considered 'heavy' as its boiling point

is above 90ºC, the margin by which it surpasses the 90ºC threshold is slight (only a few degrees at STP). Heavy volatiles are especially abundant, with a variety of structures and organoleptic properties. Some notable entries include ethyl thioacetate (**9**), dimethyl disulfide (**10**) and other alkyl disulfides, dimethyl trisulfide (**11**), dimethyl sulfoxide (**12**), 2-mercaptoethanol (**13**) and other mercaptoalcohols, esters of sulfides like 3-methylthiopropyl acetate (**14**), and various heterocyclic species such as 2-methyltetrahydrothiophenone (**15**), and 5-(2-hydroxyethyl)-4-methylthiazole (**16**), to name a few. Sensory thresholds of some of these compounds are given in Table 1.1.

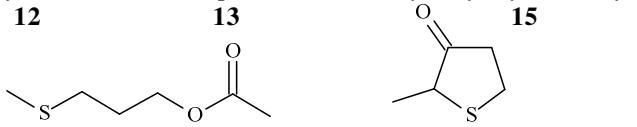
Ethyl Methyl Sulfide **5** | Carbon Disulfide **6** | Carbonyl Sulfide **7** | Methyl Thioacetate **8**



Ethyl Thioacetate **9** | Dimethyl Disulfide **10** | Dimethyl Trisulfide **11**



Dimethyl Sulfoxide **12** | 2-Mercaptoethanol **13** | 5-(2-Hydroxyethyl)-4-methylthiazole **15**



3-Methylthiopropyl Acetate **14** | 2-Methyltetrahydrothiophenone **16**

Distinction between light and heavy VSCs will play a key role in analytical methods. Often the same method cannot effectively evaluate content of both light and heavy volatiles. For many analyses, separate steps must be taken to

extract, precondition, or derivatize certain compounds in order to ensure their measurability. There is some margin, though, where heavier compounds with relatively low molecular weights can be analyzed in the same assay as lighter compounds. For instance, ethyl thioacetate can be measured simultaneously with dimethyl sulfide, ethanethiol, and methyl thioacetate [28]. Nevertheless, it is germane to examine analytical methods of these compounds based on their light and heavy character.

Table 1.1: Sensory Thresholds of Some VSCs found in Wine[29-31]

Compound	Threshold value (ppb)			Aroma description
	Wine		12%EtOH (aq)	
Hydrogen sulfide	0.001-150*		0.8	Rotten egg, decaying
	40-100**			seaweed, rubbery
Methanethiol	1.72-1.82	(red)	0.3	Rotten cabbage, cooked cabbage, burnt rubber, pungent, putrefaction
Ethanethiol	1.1	(white)	0.1	Onion, rubber, fecal, burnt match, earthy,
	0.19-0.23	(red)		durian
Carbon disulfide	30	(white)		Rubber, choking repulsive, cabbage, sulfidy
Dimethyl sulfide	10-160		5-10	Cabbage, asparagus,
	25	(white)		cooked corn, truffles, vegetal, molasses,
	60	(red)		black olive
Diethyl sulfide	0.92-18		6	Garlic, onion, cooked vegetables, rubbery,
	0.92	(white)		fecal
Dimethyl disulfide	20-45		2.5	Cabbage, cook
	29	(white)		cabbage, onion-like
	11.2-23.6	(red)		

Table 2.1 (continued): Sensory Thresholds of Some VSCs found in Wine[29-31]

Diethyl disulfide	4.3-40 4.3 1.4-2.2	 (white) (red)	20	Garlic, onion, burnt rubber
Dimethyl trisulfide			0.005-0.01	Beany
Methyl thioacetate				Sulfurous, rotten vegetables, cheesy, onion, burnt
Ethyl thioacetate				Sulfurous, cheesy, onion, burnt
Methionol	1200-4500			Raw potato, soup-like, meat-like
Methional			50	Onion, meat, mashed potato, soup, bouillon
Benzothiazole	24 50-350		50	Rubber
2-Mercaptoethanol	130-10000		1000-10000	"Boxer," poultry, farmyard, alliaceous
4-Methylthio-1-butanol	100		80-1000	Chive, garlic, onion, earthy, alliaceous

*Aroma threshold

**Flavor threshold

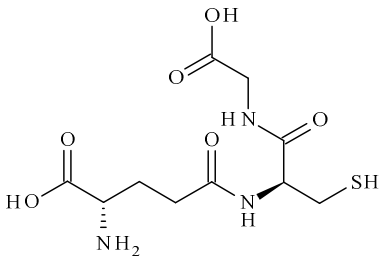
Formation of Light Volatile Sulfur Compounds in Wine

The evolution of hydrogen sulfide and dimethyl disulfide has been well-researched [32-42]. While several factors play an integral role in the production of hydrogen sulfide, ultimately its liberation relies on yeast metabolism of sulfur-containing precursors. For many years when strong, scientific wine research was still relatively nascent, a widely accepted and assumed precursor to hydrogen sulfide was elemental sulfur sprayed onto the grapes during the growing season. Elemental sulfur proves to be a highly effective antimicrobial and antifungal agent, and from the 1960s through 80s many papers were published examining the role of elemental sulfur residue in hydrogen sulfide production. Preliminary results suggested this was a probable cause for high amounts of hydrogen sulfide, as laboratory tests with sulfur-supplemented synthetic must fermentations produced observable hydrogen sulfide. Acree *et al.* [43] observed in 1972 that synthetic musts with a 10 mg/L sulfur addition suffered from high hydrogen sulfide production, more so than a similar synthetic must with sulfate addition. They measured hydrogen sulfide production with a cadmium hydroxide trap and methylene blue; a nitrogen stream displaced dissolved hydrogen sulfide into the trap and resulted in a colorimetrically measurable result. Schütz and Kunkee [32] measured hydrogen sulfide formation in 1977 using both a lead-acetate-soaked cellulose system borrowed from Rankine [44], and a sulfur-specific ion-selective electrode. The lead acetate method is subject to some criticism [37], however, as the method is considered highly inaccurate and qualitative, relying on visual ascertainment of hydrogen sulfide levels based on black color formation in the system. Furthermore, Thomas *et al.* [45] examined specific concerns about the amount of sulfur added to the synthetic musts in past experiments. In 1993 they published a method for determining sulfur residue on the grape berries,

involving washing the residue off of whole clusters using Tween 20, and analyzing the wash solution for sulfur using vacuum inductively-coupled plasma (ICP) spectrometry. This led to a realization of relatively low amounts of elemental sulfur residing on grapes within days after dusting in the vineyard. Average values of 1-3µg/g berry weight, which translates to roughly 1.2-3.4 mg/L juice, were found across several vineyards, paling in comparison to the average analyses of 10-100mg/L of several preceding studies. This led to a repeat of Acree's [43] experiment utilizing a cadmium hydroxide trap, but with the lower concentrations of elemental sulfur measured on the grapes (0, 1.7, and 3.4 mg/L) [37]. Conclusions from this study confirmed suspicions of previous reports, ultimately showing the lack of significance of even the largest (3.4mg/L) amount of elemental sulfur found on the grapes. Thus, hydrogen sulfide production was attributed to yeast manipulation of other precursors.

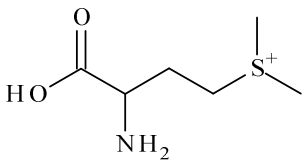
Other factors which may have a hand in hydrogen sulfide production include a pantothenate deficiency [33, 46, 47], levels of glutathione (**17**) in the yeast [33], and reduction of both (or either) sulfate and/or sulfite [36, 43, 47]. Spiropolous *et al.* [47] have suggested that the underlying issue in all situations is that of nitrogen levels, specifically of both easily assimilable amino acids and sulfur-containing amino acids. In reviewing yeast metabolism and assimilation of nitrogen sources, levels of cysteine and methionine, which tend to suppress sulfate and sulfite reductases, are in balance with important non-sulfur-containing amino acids, which may or may not (depending on certain conditions) suppress these enzymes. Without delving as deeply into the enzymology and genetics of their research, some influencers of hydrogen sulfide production, namely sulfate and sulfite, are merely extensions of a latent nitrogen-related influencer. Readers are directed toward a great review of these findings in reference [47]. These factors are highly complex, and even

such a complex solution cannot encompass the entirety of chemical behavior among grapes, must, and yeast. A single, unified, comprehensive explanation of hydrogen sulfide formation in wines is unlikely.



Glutathione
17

Dimethyl sulfide also receives considerable attention for its presence (in most respects unwanted) in many wines [25, 35, 38, 42, 48, 49]. Believed to also stem from sulfur-containing amino acid precursors, its formation is similarly esoteric. De Mora *et al.* [50] performed a radiolabeling experiment with ^{35}S-cysteine, and confirmed a pathway of dimethyl sulfide formation. However, these findings were related to yeast contact with the wine and presence in yeast lees after racking. Commonly dimethyl sulfide off-odors are generated during bottle-aging, without lees contact [51, 52]. This led Segurel *et al.* [49] to investigate potential precursor compounds, and design a metric for potential dimethyl sulfide (P-DMS). In examining various candidates, they found S-methylmethionine (**18**) to produce reasonable levels of dimethyl sulfide during a heat-alkaline synthetic aging trial. Still, the absolute cause of dimethyl sulfide liberation post-bottling is not clearly understood.



S-Methylmethionine
18

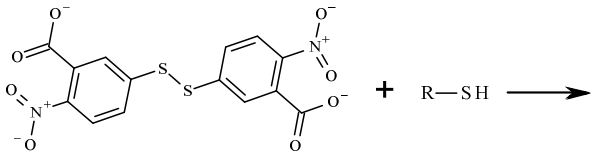
Analysis of Lighter Sulfur Volatiles

One difficult aspect about light sulfur volatiles is, by definition, their volatility. Precautions must be taken to procure accurate measurements. Past enlightening studies have revolved around analysis as a response to sensorial input; a foul off-odor is noticed in several wines, and the bottles are passed on to an analyst. In many of these studies, the most common compounds are those mentioned above; hydrogen sulfide, dimethyl sulfide, carbon disulfide, and the small thiols including methanethiol and ethanethiol. Among these, disulfides are also present (or subsequently formed) by the oxidation of said thiols. As mentioned, methyl and ethyl thioacetates are also appropriately studied with other smaller VSCs. Several methods have been used to quantify these compounds, and important submissions will be outlined.

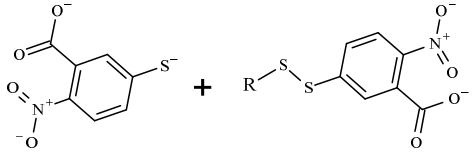
Sample Preparation

A common assay in biological [53] and food [54, 55] systems for various smaller thiols involves a chromophore-based compound called 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB, **19**), or Ellman's reagent [56]. This specific compound relies on a highly electronically withdrawn disulfide bond to react (eq. 1) with smaller, nucleuophilic thiols, leaving a dianion chromophore (2-nitro-5-sulfido benzoate, **20**), and its alkyl-disulfide analogue.

The production of said chromophore imparts a yellow color to the solution (the remaining disulfide analogue is colorless), which can be measured spectrophotometrically by UV-VIS spectrometry. Because wine pigments would convolute UV-VIS readings, however, this method only proves useful for verification of concentrations of prepared standards, and not direct wine analysis [57]. Measured amounts of a single thiol are placed in a pH 7 solution (using a phosphate buffer) with DTNB and the resultant yellow color is calibrated and measured at 412nm. This method is useful in preparatory stages for accurate measurement of volatile thiols [57]. However, due to the impartial nature of DTNB, this method offers little aid in simultaneous analysis of multiple thiols.



5,5'-Dithiobis(2-nitrobenzoic Acid)
19



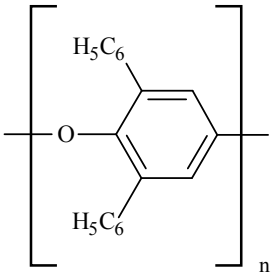
2-Nitro-5-sulfido Benzoate
20

(1)

Some small thiols are highly reactive in the presence of certain species. Transition metals, for instance, even in trace amounts, can catalyze oxidation of thiols into disulfides [38, 58]. Sampling containers must also be considered. Generally direct gas analyses of sulfur mixtures involved storage in poly(vinyl fluoride) bags to ensure chemical inertness [59]. Glass vials, commonly used

for storage and sampling, contain relatively active hydroxyl groups on the surface. Additional precautions must be taken to deactivate the surfaces of these vials. Common treatment is deactivation using trimethylchlorosilane, dimethyldichlorosilane, methyltrichlorosilane, and hexamethyldisilazane [60]. Cleaning glassware with a 5% solution in toluene, hexane, or dichloromethane will replace the hydroxyl groups with silyl ether groups.

Volatile sulfur compounds can be separated and analyzed by gas chromatography. Due to the low concentration in wine, further concentration is necessary. The purge-trap enrichment method can be used to improve the sensitivity of detection. Poly(2,6-diphenyl-*1-4*-phenylene oxide) (**21**), also known as Tenax™, was used for its high thermo-stability required for thermal desorption [61]. The volatile sulfur compounds can also be cryogenically trapped [4, 57] on a small portion of the capillary column submerged in liquid nitrogen. The frozen material trapped in the column is re-volatilized after the trapping.



Poly(2,6-diphenyl-*1-4*-phenylene Oxide)
"Tenax"
21

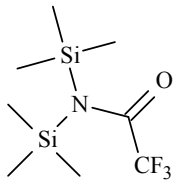
Presently, the most common method of volatile sulfur analysis does not function on removal of unwanted compounds, but rather the selectivity of sulfur compounds for analysis. Mestres *et al.* [62] used head space solid phase micro-extraction (HS-SPME) to analyze volatile sulfur compounds in wine. Polyacrylate (PA) and poly(dimethylsiloxane) (PDMS) fibers, as well as a bi-layered activated carbon/PDMS fiber in a later study [63], have been evaluated for the extraction efficiency of volatile sulfur compounds. These studies proved highly enlightening. PDMS, which is significantly less polar than polyacrylate and prefers moderate- to non-polar compounds, proved more effective in extracting some VSCs, namely ethylmethyl sulfide, diethyl sulfide, and methylpropyl sulfide, but showed little advantage (or disadvantage) in extracting dimethyl sulfide, methyl and ethyl thioacetates, carbon disulfide, and other alkyl disulfides. However, smaller volatiles like hydrogen sulfide, methanethiol, and ethanethiol were not examined. The activated carbon/PDMS fiber showed a considerable affinity for sulfur compounds, and has since been adopted as the standard for HS-SPME VSC analysis. Recently, reports of three-phase fibers coated with activated carbon/PDMS/poly(divinylbenzene) (DVB) used for larger, heavy thiol derivatives have surfaced, which will be discussed later.

Increased polarization of the sample liquid *via* addition of sodium chloride effectively increases partition coefficients at the gas-liquid interface, and improves extraction. However, some of the most volatile compounds showed the least absorption by the SPME fiber. This phenomenon has been attributed to competitive absorption by which larger, less volatile compounds displace more volatile compounds and consume more space on the fiber [28, 59, 62, 64]. For this reason, shorter extraction times are generally preferred, at the sacrifice of proper equilibrium. Activated carbon phases somewhat

compensate for this problem due to its pore structure, which can detract from displacement by groups too large to fit in smaller crevices. Murray [59] has criticized activated carbon/PDMS fibers, citing that other compounds like carbon disulfide can interfere with other molecules' ability to bind to the fiber, even if not by a competitive mechanism. The mere presence of carbon disulfide can reduce the accuracy of other VSCs like dimethyl sulfide. Mestres' group also noted the decomposition and artifact formation with high-temperature extractions, suggesting a 30ºC was optimal.

Artifact formation was further addressed by Fang and Qian [28], concurring with low extraction temperatures and suggesting a deactivation step for the injector using N,N-bis(trimethylsilyl)-trifluoroacetamide (**22**) and deactivation of sample vials. It is also common practice to flush all sample vials with nitrogen or argon to avoid any oxidation and disulfide artifact formation [28, 65, 66]. Lastly, a precautionary measure should be taken to eliminate the activity of metal ions present in the system, as such are known to catalyze thiol oxidation as mentioned. Addition of EDTA or other organic acids such as citric acid and malic acid [28, 65-67] will chelate trace metals in solution and prevent catalysis.

Extraction is further improved by agitation, increased headspace, and dilution of ethanol. Still, the matrix effect surmises one of the greatest challenges of wine sulfur analysis, due to the great variability of wine (and wine-based products like Cognacs and brandies), and remains as a major challenge [58, 62, 63, 68-72].



N,N-bis(Trimethylsilyl)-acetamide
22

Separation

Originally researchers used dimethylpolysiloxane columns for separation *via* GC, which vary in composition and thickness. Some have utilized non-polar PDMS (DB-1) [39, 58], or PDMS fluid (HP-101) [36] columns. Slighter higher polarities are reached through partially-substituted PDMS with phenyl (DB-35) [73], or cyanopropyl groups (DB-1701) [74]. Specific sulfur columns (SPB-1, Figure 1.1) have also been used successfully [63, 72], which rely on a very thick film (4 μ) of PDMS to retain highly volatile compounds [60]. Others have used packed columns [48, 75], but the most common used for sulfur analysis are polar poly(ethyleneglycol)-based (wax) columns [28, 30]. Good separation is achieved by Qian's group using a 2-nitroterephthalic acid-substituted wax column (FFAP, Figure 1.2)(Qian, unpublished chromatogram). Siebert *et al.* [76] have also shown effective separation with a dual-column approach (Figure 1.3), using serially connected wax and (5%phenyl)-PDMS (VB-5) columns with a 2m retention gap.

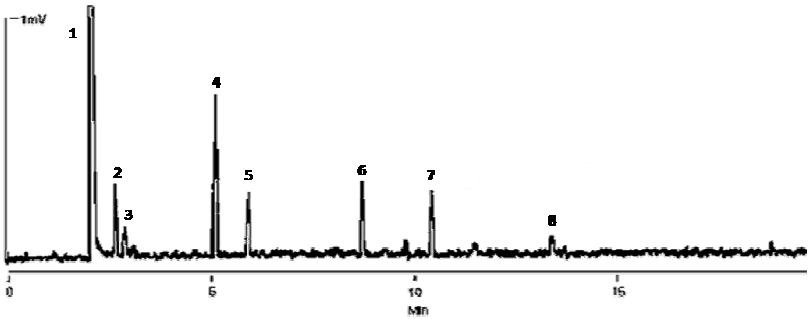


Figure 1.1: Chromatogram of sulfur analysis of sour natural gas using SPB-1 column (Courtesy of Supelco, Bellafonte, PA). 1. H_2S, 2. COS, 3. SO_2, 4. DMS, 5. MeSH, 6. EtSH, 7. Isopropanethiol, 8. *Sec*-Butanethiol.

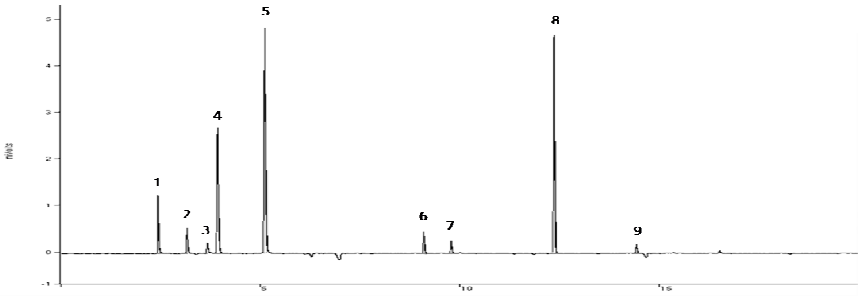


Figure 1.2: Chromatogram of sulfur analysis of Chardonnay using DB-FFAP column (Qian, unpublished chromatogram). 1. H_2S, 2. MeSH, 3. CS_2, 4. DMS, 5. EMS (IS), 6. MeSOAc, 7. EtSOAc, 8. DIDS (IS), 9. DMTS.

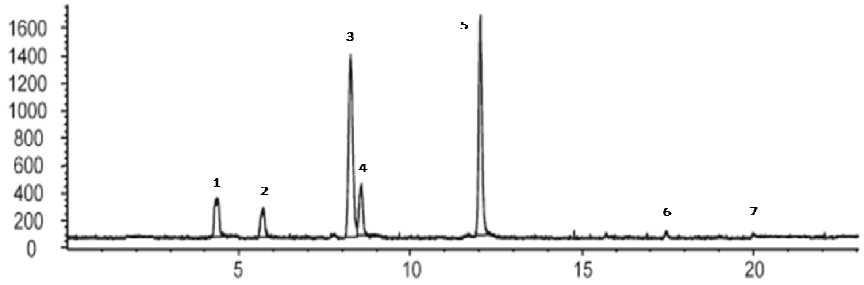


Figure 1.3: Chromatogram of sulfur analysis of white wine using VFWAXms to VB-5 dual column (reproduced with permission from reference [76], copyright 2010). 1. H_2S, 2. MeSH, 3. DMS, 4. CS_2, 5. EMS (IS), 6. MeSOAc, 7. EtSOAc.

Temperature programs are often used, generally ramping from 35-60ºC to 150-300ºC in anywhere from 5 to 30 minutes [30]. A common nuisance in wine matrices is the large peak from sulfur dioxide, a compound utilized at multiple stages in winemaking [28]. The large, wide peak eclipses more pertinent, odor-active thiols; thus, its removal is critical. Simple addition of acetaldehyde can solve this issue [28] (Figure 1.4), as a known affinity between the two compounds exists [77]. Other aldehydes can also be used to eliminate the interference of SO_2 in wine.

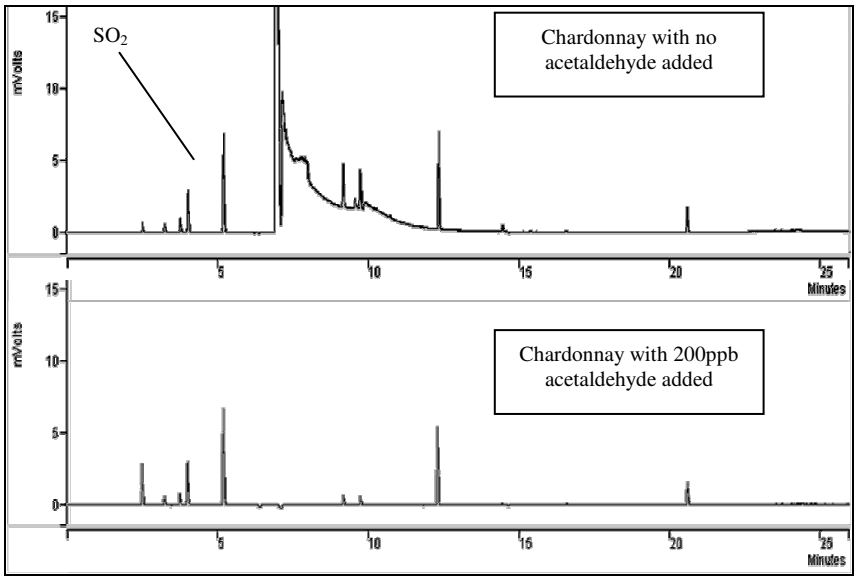


Figure 1.4: Chromatograms showing the effects of acetaldehyde on SO_2

(Unpublished chromatogram from Qian's Laboratory)

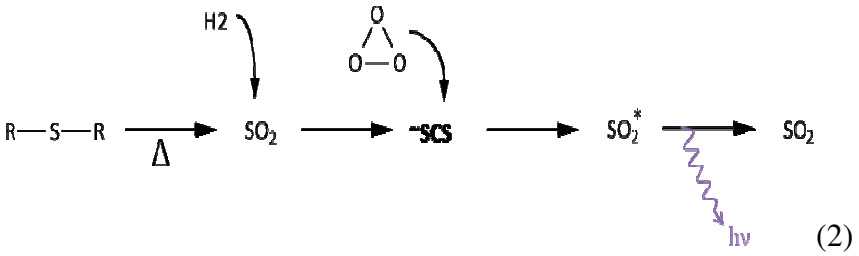
Sulfur Detectors

Both flame ionization detection (FID) and mass spectrometry (MS) have been used to quantify VSCs in wine. However, its detection limits are generally too poor to analyze sulfur in wine samples. Both FID and MS have been overshadowed by sulfur-specific detection methods.

Sulfur Chemiluminescence Detector

Sulfur chemiluminescence detectors (SCDs) are popularly used for volatile sulfur detection in wines due to their high sensitivity and equimolar response functionality. The principle behind the system involves decomposition of sulfur molecules (equation 2) into sulfur dioxide, which then reacts with hydrogen to produce a sulfur chemiluminescent species, notated

here as ~SCS, the details of which are somewhat unclear [78]. However, it is accepted that this chemiluminescent species reacts with ozone to produce an excited state of sulfur dioxide, SO_2*, which relaxes to yield a spectrum focused around 380nm. The response functionality is dependent on the concentrations of ozone and ~SCS, meaning a constant overabundance of ozone would create a first-order, linear relationship between response and concentration: response=kC [79]. SCDs also often incorporate integrated FIDs, though this has been known to cause problems involving the transfer line temperature between the two detectors. Some other downsides of the SCD are the cost and maintenance as certain maladies like probe alignment can result in a finicky system [30].



(2)

Atomic Emission Detector

Much like those of trace metal analysis, atomic emission detectors can be used to analyze sulfur compounds based on specific sulfur-atom emission spectra. The technique, though still in use, was never quite as popular for wine analysis as some others. Atomic emission is a universal detection mechanism, keying in on specific emission spectra unique to each atom's excitation-relaxation cycle. Samples are volatilzed and atomized *via* heat source, and

individual atoms are excited (S*), releasing photons in relaxation. The method is sulfur-specific by measuring emission of wavelength 132nm or 181nm [80, 81]

Flame Photometric Detector

Flame photometric detectors (FPDs, Figure 1.5) are similar to FIDs, but rely on an excitation-relaxation photon emission stimulated in lieu of ionization. Within the flame, sulfur species become oxidized and react (equation 3) to form the excited sulfur dimer species, S_2*, which relaxes to S_2, emitting a photon at 394nm. The photon is then absorbed by a photomultiplier in the detector, offering exceedingly low detection limits [82]. FPD was the most popular detector for some time [24, 55, 64], though some limitations were well-noted. Specifically, introduction of hydrocarbon species co-eluting with sulfur species is known to cause quenching (equation 4) of the response [83]. As hydrocarbons are burned by the flame, carbon monoxide is produced which chemically interacts with the stimulated S_2* units and lessens the observed signal. Responses are based on the number of sulfur atoms present in each compound, with quadratic functionality: response=kC^b. Generally the b value ranges from 1.5 to 2. Calibrations thus involve logarithms of analyte/internal standard ratios, comparing peak areas and peak heights [24, 62].

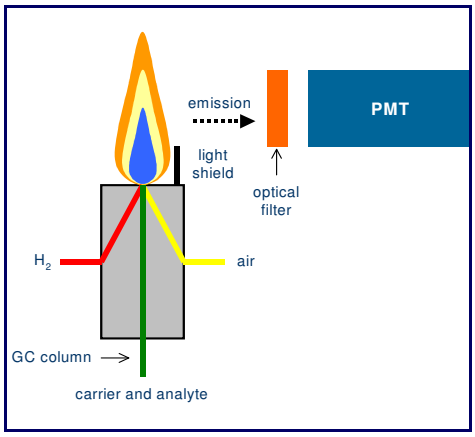
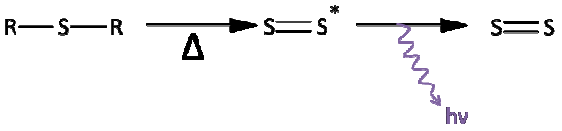


Figure 1.5: Schematic of flame photometric detector (Courtesy of Hewlett-Packard Co., Analytical Customer Training, Atlanta, GA.)



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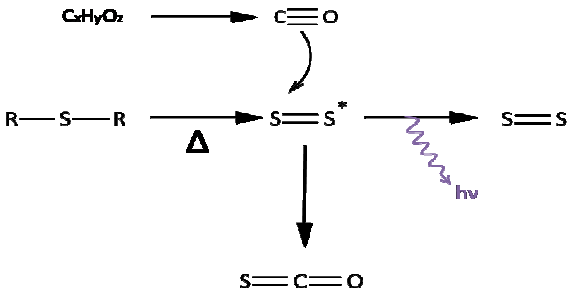


(4)

Pulsed-flame Photometric Detector

In an effort to overcome issues with sensitivity in traditional FPDs, the pulsed-flame photometric detector (PFPD, Figure 1.6) forgoes a constant, steady flame in favor of a punctuated mechanism. Effectively, hydrocarbons, carbon monoxide, carbon dioxide, and sulfur dioxide have different relaxation patterns based on time. The former three relax much more quickly (2-3ms) than sulfur dioxide (5ms), and this emission lag is utilized to focus strictly on sulfur species. The PFPD works just like a FPD, allowing a buffer time amidst the pauses between flame pulses to ignore early emissions from C and other atoms and greatly increase sensitivity [83, 84]. This detector has been popularized due to its high selectivity and reproducibility, if slightly less sensitive than SCD [28, 69].

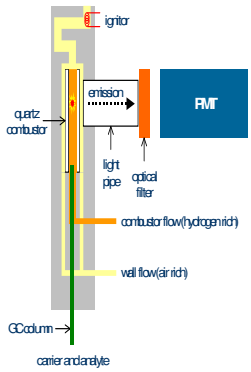
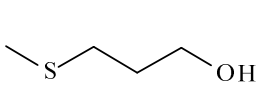


Figure 1.6: Schematic of pulsed flame photometric detector (Courtesy of Varian Inc., Palo Alto, CA)

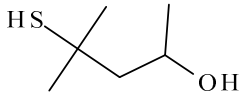
Formation of Heavy Volatile Sulfur Compounds in Wine

Some larger, heavy volatiles, as discussed, can often impart beneficial flavor to wines. 3-Mercaptohexan-1-ol and its acetate ester are known to impart tropical, passion fruit, grapefruit, and guava aromas to wine [13, 30]. Such heavy volatiles are essential to varietal aromas of white wines, notably Sauvignon Blanc [27, 39, 85] and Muscat [34, 86]. Several prize-winning rosé wines from Provence were found to contain both aforementioned mercaptohexyl compounds at concentrations above their thresholds, attributing as well to varietal aroma [87]. Origins of some heavy thiols involve reactions of amino acid methionine and ethanol. Well-known VSC methionol (3-methylthiopropan-1-ol, **23**) and other C_3 sulfur compounds are believed to originate in this manner. Other larger forms are found as conjugate species with amino acid cysteine, which are cleaved enzymatically during fermentation. A β-lyase enzyme present in the yeast liberates bound thiols and contributes to the fermentation bouquet [88]. This explains why such sulfur compounds, despite their low thresholds, are not immediately detectable in the grapes. However, anecdotal reports for many years have documented the 'Sauvignon-blanc-like' aftertaste that arises 30 seconds after consumption of the grapes. This is believed to be a retro-olfactory phenomenon caused by compounds hewn from their precursors by mouth enzymes [89]. Within the grape, Peyrot des Gachons *et al.* [90] have shown that, while 4-mercapto-4-methylpentan-2-ol (**24**) and its ketone analogue are equivalent in the skins and juice, 3-mercaptohexan-1-ol is considerably more present in skins. Thus more extraction can be achieved by extended maceration. However, other factors will affect its retainment in red wines; oxygen and phenolic compounds greatly reduce the presence of 3-mercaptohexan-1-ol over time, though sulfur dioxide, and to a lesser extent anthocyanins, can offer protection. Dozens of other

heavy volatiles exist in wines [30]. Because of their lower volatility and concentrations, heavy VSCs have generally been analyzed differently than lighter forms.



Methionol
23

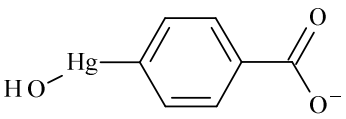


4-Mercapto-4-methylpentan-2-ol
24

Analysis of Heavy Sulfur Volatiles

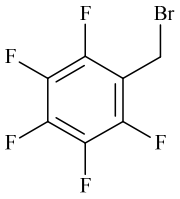
Sample Preparation

The standard method for analyzing heavy VSCs was pioneered by Tominaga *et al.* in 1998. [19, 27, 90-94]. Preparation begins with a solvent extraction at neutral pH, generally in dichloromethane, ethyl acetate, Freon 11, or any combination thereof. The organic phase is centrifuged and separated, then further extracted with *p*-(hydroxymercuri)benzoate (**25**). This derivatization reagent preferentially binds to sulfur species, and allows the bound conjugates to adhere to a strong anionic exchange column for concentration and washing of unwanted material. To liberate the thiols from the column, an abundance of larger thiol compounds like cysteine or glutathione can replace the analytes. The heavy-thiol-rich eluate is then extracted again with dichloromethane before analysis by GC.

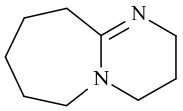


p-(Hydroxymercuri)benzoate
25

Recently, Rodríguez-Bencomo *et al.* analyzed larger thiols through the use of SPME and alternate derivatization [70]. In their method, wines are extracted and similarly loaded onto solid phase extraction cartridges containing styrene divinylbenzene phases. The compounds are derivatized with pentafluorobenzyl bromide (**26**) with the aid of strong alkaline agent 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, **27**), and washed with mercaptoglycerol. For the SPME fiber, a triple-phase activated carbon/DVB/PDMS coating was used to improve extraction specifically of the fluorobenzyl conjugates. SPME analysis of the derivatized forms proved promising [70]. This method was adapted from a previous work by Mateo-Vivaracho *et al.* [95], who used the derivatized species for direct injection into GC-MS.



Pentafluorobenzyl Bromide
26



1,8-Diazabicyclo[5.4.0]undec-7-ene
27

Sample Analysis

For the most part, the difference in heavy and light volatile sulfur analysis is comprised of the preparatory measures necessary for proper extraction. The basics of separation (GC) and detection (most often MS) have been adequately discussed, and are applicable for heavier volatiles. Mass Spectrometry seems to gain greater preference for heavier compounds; however, pretreatment of samples must be done to concentrate the VSCs to meet systems' detectability.

EFFECTS OF ETHANOL CONCENTRATION IN WINE ON ADSORPTION OF VOLATILE SULFUR COMPOUNDS ON SPME FIBER

Peter M. Davis and Michael C. Qian

Abstract

Complications in the analysis of volatile sulfur compounds in wine using solid-phase microextraction (SPME) arise from sample variability. Constituents of the wine matrix, including ethanol, affect volatility and adsorption of sulfur volatiles on the SPME fiber, which can impact sensitivity and accuracy. Several common wine sulfur volatiles, including hydrogen sulfide (H_2S), methanethiol (MeSH), dimethyl sulfide (DMS), dimethyl disulfide (DMDS), dimethyl trisulfide (DMTS), diethyl disulfide (DEDS), methyl thioacetate (MeSOAc), and ethyl thioacetate (EtSOAc), were analyzed using SPME-GC equipped with pulsed-flame photometric detection (PFPD) at various ethanol concentrations in a synthetic wine matrix. Ethyl methyl sulfide (EMS), diethyl sulfide (DES), methyl isopropyl sulfide (MIS), ethyl isopropyl sulfide (EIS), and diisopropyl disulfide (DIDS) were tested as internal standards. The absorption of volatile compounds on the SPME fiber is greatly affected by ethanol. All compounds exhibit a stark decrease in detectability with the addition of ethanol, especially between 0.0 and 0.5%v/v. However, the ratio of interested sulfur compounds to internal standard becomes more stable when total alcohol concentration exceeds 2%. EMS was found to best resemble DMS; EIS and DES were found to best resemble DMDS, MeSOAc, and EtSOAc; DIDS was found to best resemble DEDS, DMTS, H_2S, and MeSH.

Introduction

Volatile sulfur compounds, often having very low odor thresholds, are responsible for many common off-flavors in wine [22]. Such compounds often have offensive odors, imparting notes of cabbage, onion, garlic, or rubber to

wines [3, 20, 22, 38, 64, 96]. However, because of their relatively low concentrations, a highly sensitive method is required to analyze accurately.

Solid-phase microextraction (SPME) is a common method for extraction of volatiles from food and beverage samples [28, 64, 81]. A coated fiber is extended into the headspace of a sample vial, allowing a finite number of volatiles to adsorb until later thermal desorption. The relatively minute scale of the fiber's capacity compared to the entire sample ensures that the removal of a small fraction of the volatile content will not disturb the sample equilibrium. This also allows for very fast equilibration between the air-fiber interface as compared to static headspace analyses [97]. However, due to the limitation of space for volatiles to adhere to the fiber, the presence of other volatiles from the matrix can interfere with analysis [60].

In wine samples, ethanol concentration is large and varied. This can cause issue with sulfur analysis, as a decrease in sensitivity has been shown in samples with increasing ethanol content [63, 98]. While fiber competition may be a factor, some have suggested that ethanol content acts as a co-solvent in the sample liquid, affecting transition coefficients at the liquid-air interface [99]. This suggestion was a result of similar ethanol-concentration studies using static headspace techniques. Furthermore, it has been shown that varying sample parameters, such as temperature, extraction time, and matrix effects can effect individual compounds differently [99]. This is of major concern for wine analysis, as parameters such as ethanol content and volatile and non-volatile profile are well-varied. For this reason, a proper internal standard must be found for each analyte, ensuring their behaviors in response to parameter variation are consistent and shared.

Carboxen-poly(dimethylsiloxane) (PDMS) is the most commonly-used fiber coating for SPME sulfur analysis. Because of its porous structure, it does

not exhibit the displacement effects of other, more uniform fibers [97]. However, it has also been shown to have less repeatability [62].

In order to investigate the effects of ethanol on SPME sensitivity, several common sulfur compounds were analyzed on three different fibers. Five internal standards were used, including the conventional ethyl methyl sulfide (EMS) and diisopropyl disulfide (DIDS). Samples were prepared with identical concentrations of analytes and internal standards, with ethanol concentration varying.

Materials and Methods

Chemicals

Sodium sulfide, methanethiol (MeSH), dimethyl disulfide (DMDS), dimethyl trisulfide (DMTS), and diisopropyl disulfide (DIDS) were from Sigma-Aldrich (St. Louis, MO, USA). Methyl thioacetate (MeSOAc), ethyl thioacetate (EtSOAc), and diethyl sulfide (DES) were from Alfa-Aesar (Ward Hill, MA, USA). Ethyl methyl sulfide (EMS), dimethyl sulfide (DMS), diethyl disulfide (DEDS), methyl isopropyl sulfide (MIS), and ethyl isopropyl sulfide (EIS) were from TCI America (Portland, OR, USA). Methanol was from EMD Chemicals Inc. (Gibbstown, NJ, USA), l-tartaric acid from J.T. Baker (Phillipsburg, NJ, USA), and ethanol was from Koptec (King of Prussia, PA, USA).

Sample Preparation

Hydrogen sulfide standards were prepared using equivalents of sodium sulfide (Na_2S) dissolved in distilled water, and further diluted with cold (-15ºC) methanol. MeSH standards were prepared by blowing the pure gas over

cold methanol and recording gained mass. All other standards were prepared by dilution with cold methanol. A standard mixture (mix 1) was prepared containing DMS (3000µg/L), MeSOAc (1285.5µg/L), DMDS (218.28µg/L), EtSOAc (564µg/L), DEDS (55.5µg/L), and DMTS (46.76µg/L). Because MeSH readily oxidizes to DMDS, and the higher affinity for DMDS on the SPME fiber causes much greater peak responses, the two compounds were not analyzed simultaneously. A separate mixture (mix 2) was thus prepared containing MeSH (36.9mg/L) and H_2S (31.25µg/L). Finally, a mixture of internal standards (IS mix) was prepared containing EMS (5mg/L), DES (1mg/L), MIS (1.5mg/L), EIS (1mg/L), and DIDS (25.9µg/L).

Samples were prepared in 20mL deactivated screw-cap glass vials with Teflon-faced silicone septa. Synthetic wine was prepared using 2ml of 3.5g/L tartaric acid solution. Ethanol was added corresponding to target concentrations of 0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, and 6.0%. Saturated salt water was then added to a total volume of 10mL in order to drive more volatiles into the headspace [97]. Vials were flushed gently with argon under low flow rate (barely disturbing the surface of the sample liquid) to avoid turbulence. All samples received 20µL of standard mixture (mix 1 or 2) and 10µL of IS mix (30µL of methanolic solutions added in total). In the case of mix 2, all standards were introduced via syringe through the sample-vial septum to avoid oxygen contact. All standards were stored in the freezer (-15ºC).

SPME Conditions

Three SPME fibers were used: 85µm Carboxen-PDMS, 65µm poly(divinylbenzene)(DVB)-PDMS, and 50/30µm DVB-Carboxen-PDMS (Supelco, Bellafonte, PA). The samples were equilibrated at 30ºC for 5

minutes and the extraction took place for 20 min with agitation at 250rpm. Injection temperatures for each fiber were 300ºC, 250ºC, and 270ºC, respectively. Samples were analyzed in triplicate.

GC-PFPD

Samples were run on a Varian CP-3800 gas chromatograph equipped with a pulsed-flame photometric detector (PFPD; Varian, Walnut Creek, CA). A DB-FFAP column (30m x 0.32mm x 1µm, Agilent, Palo Alto, CA) was used for separation. A temperature program was used for the GC oven: 35ºC for 3min, ramped to 150ºC at 10ºC/min, held 5min, ramped to 220ºC at 20ºC/min, held 3min. Nitrogen was used as carrier gas at 2mL/min flow rate. Detector temperature was 300ºC with 14mL/min hydrogen, 17mL/min air 1, and 10mL/min air 2. The PFPD was operating in sulfur mode, with 6ms gate delay and 20ms gate width. Data analysis relied on square roots of peak areas.

Results and Discussion

Direct Analysis

In all cases, sensitivity was seen to decrease with increased ethanol content. A very stark decrease was seen immediately with the addition of ethanol (0-0.5%) on Carboxen-PDMS (figure 2.1) and DVB-Carboxen-PDMS fibers (figure 2.2). Results from DVB-PDMS fiber are shown in the appendix. DMS, DMDS, MeSOAc, EtSOAc (figure 2.1B, 2.2B, 2.3B), as well as internal standards EMS, DES, MIS, and EIS (figures 2.1A, 2.2A, 2.3A) all exhibited similar curve shapes. DEDS, DMTS, and DIDS (figures 2.1C, 2.2C, 2.3C) shared a different shape, with more gradual decrease with respect to

concentration. Methanethiol shows a decrease, but with a more gradual curve like those of larger species. Hydrogen sulfide exhibits very little influence from ethanol concentration, possibly suggestion the ability to enter very small pores on the Carboxen fiber phase and avoid competition (figures 2.1D, 2.2D, 2.3D).

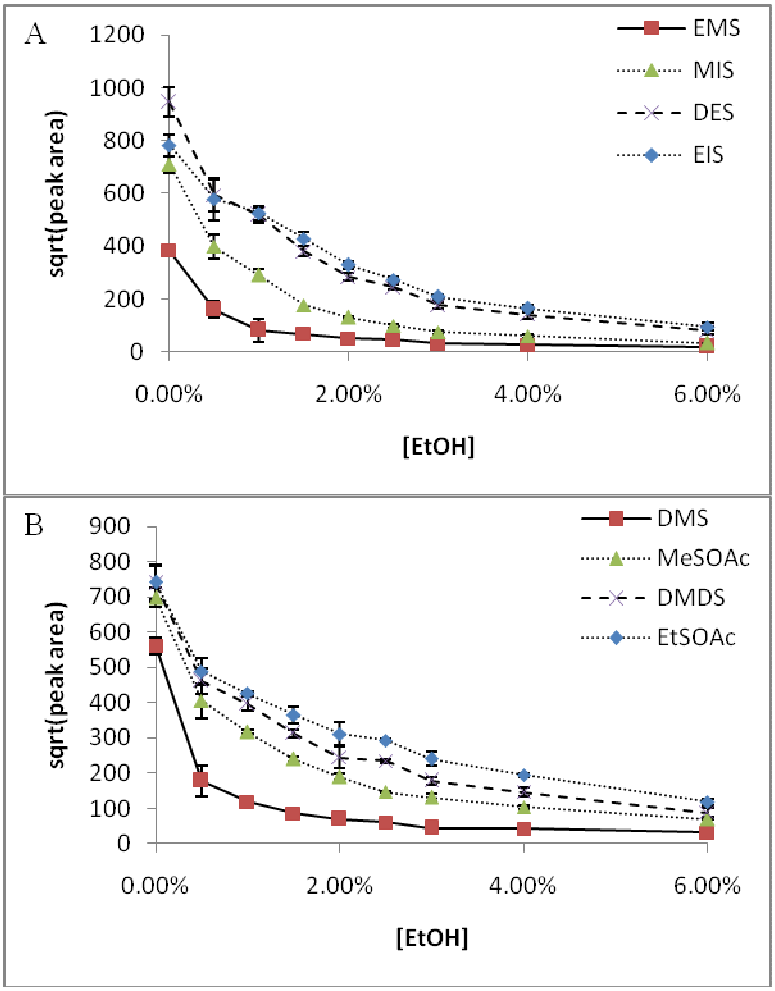


Figure 2.1: Effects of ethanol on adsorption on Carboxen-PDMS SPME fiber of: A) internals standards EMS, MIS, DES, and EIS; B) DMS, MeSOAc, DMDS, and EtSOAc

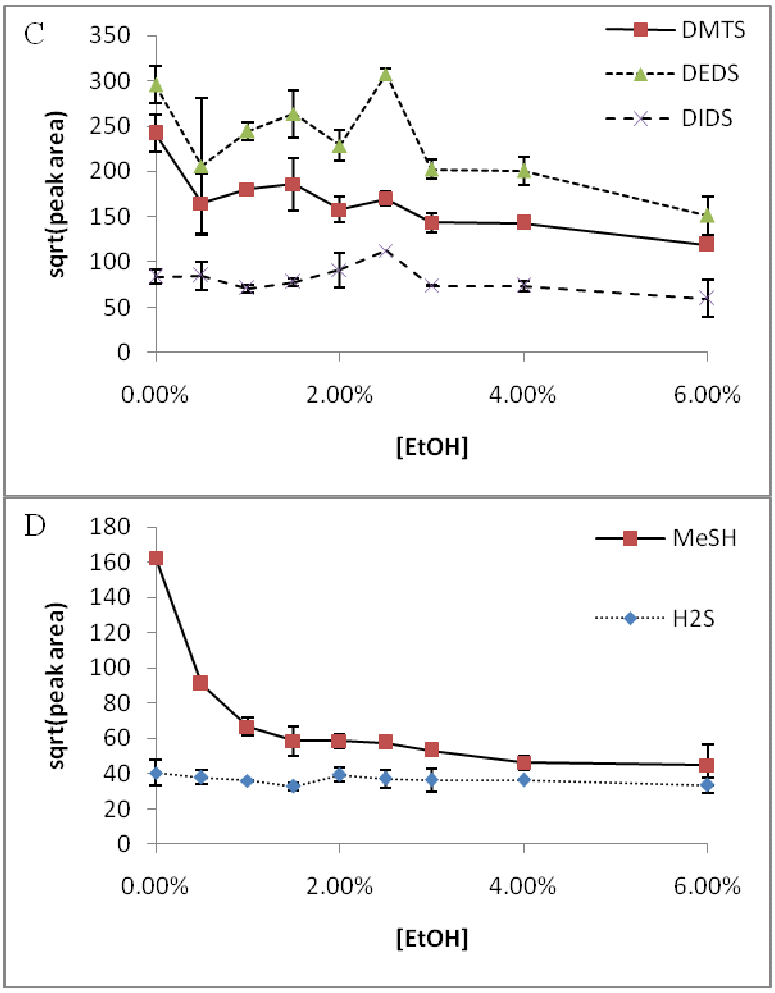


Figure 2.1 (continued): Effects of ethanol on adsorption on Carboxen-PDMS SPME fiber of: C) large compounds DMTS, DEDS, and DIDS (IS); D) highly-volatile compounds MeSH and H_2S

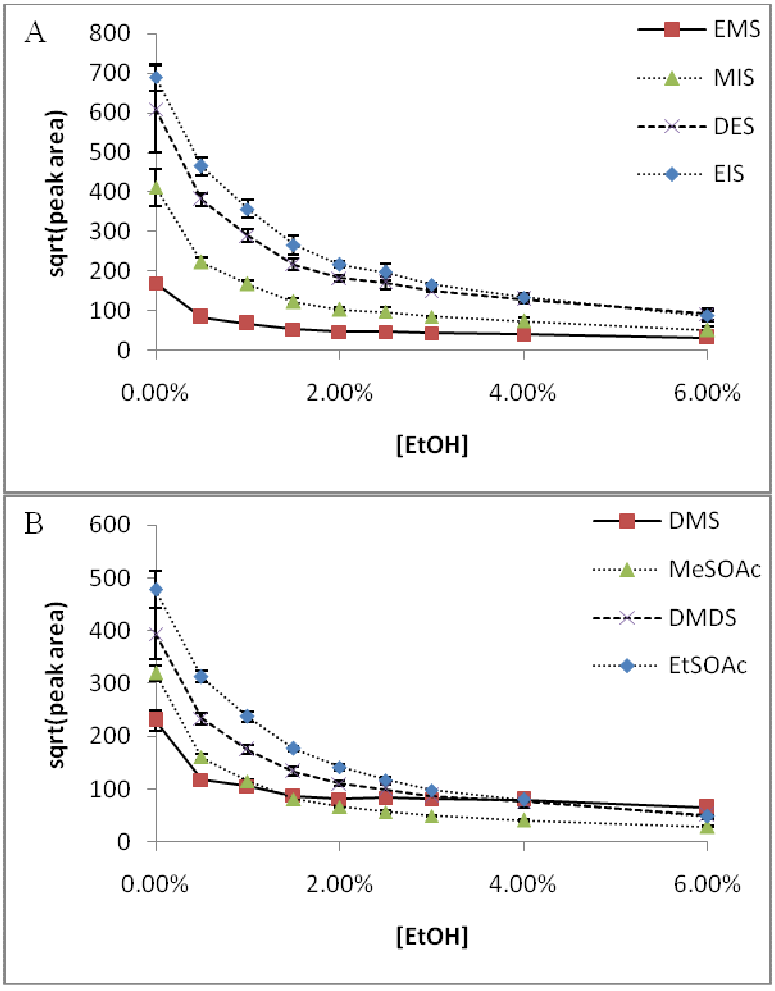


Figure 2.2: Effects of ethanol on adsorption on DVB-Carboxen-PDMS SPME fiber of: A) internals standards EMS, MIS, DES, and EIS; B) DMS, MeSOAc, DMDS, and EtSOAc

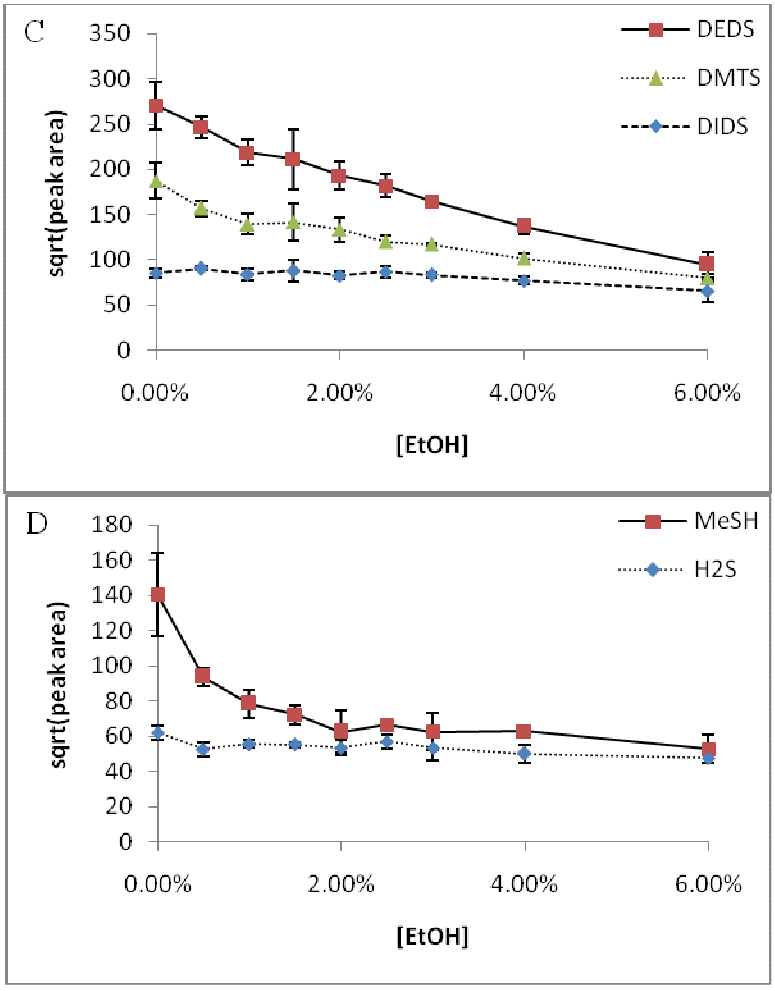


Figure 2.2 (continued): Effects of ethanol on adsorption on DVB-Carboxen-PDMS SPME fiber of: C) large compounds DMTS, DEDS, and DIDS (IS); D) highly-volatile compounds MeSH and H_2S

The DVB-PDMS fiber showed less sensitivity toward all compounds, but also less dependence on ethanol concentration (figure 2.3), as has been previously noted [62]. The smaller compounds and internal standards (save DIDS) show a less-sudden decrease with ethanol, almost linearly (figure 2.3A, B). Larger compounds like DEDS show a gradual decrease, while DMTS and DIDS are barely affected (figure 2.3C). Hydrogen sulfide and methanethiol show no signs of significant change above 0.5% ethanol (figure 2.3D). These phenomena may be indication of a more adsorptive mechanism, while the carboxen allows a more absorptive mechanism [97]. This is due to the uniform structure of solid microspheres within the DVB phase, compared to the non-uniform porosity of activated charcoal.

Ratio Analysis

It is shown that different sulfur compounds are not affected by ethanol concentration in the same way. This causes an issue with wine analysis, where two different wines could have up to 20-30% difference in ethanol concentration, more so if brandies or other spirits are involved. However, calibration and quantification rely on the ratio of analytes to internal standards. If an internal standard is selected that most closely mimics the behavior of an analyte in question, its ratio, at constant concentrations of both, will be constant despite ethanol increase. Thus, proper internal standards are necessary for accurate quantification.

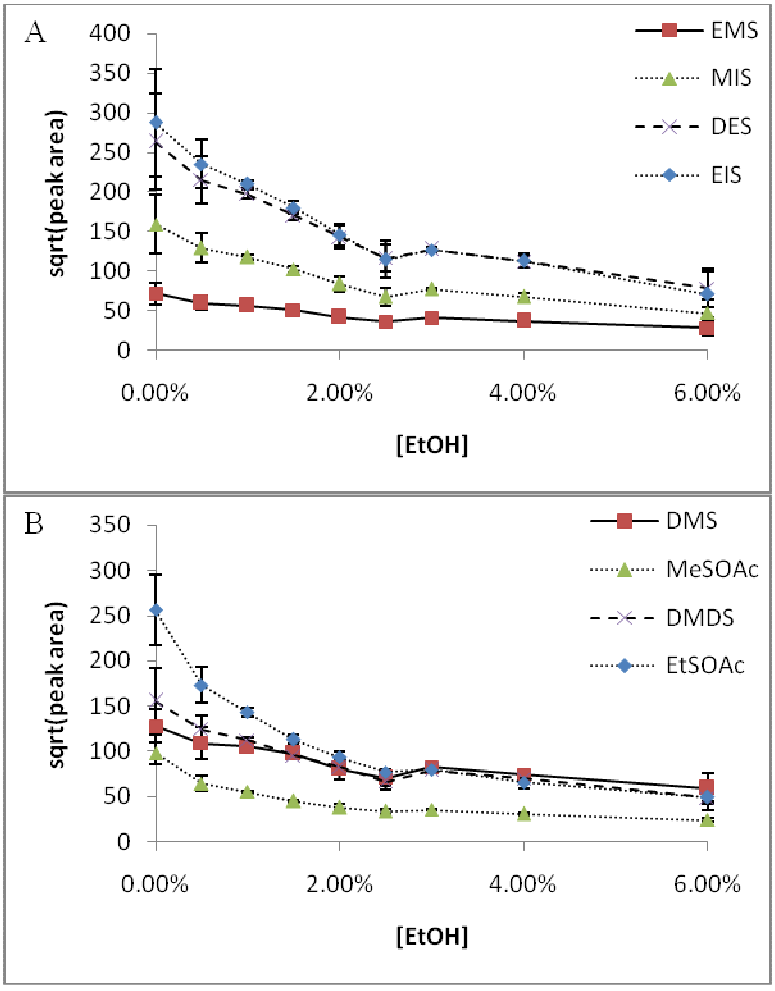


Figure 2.3: Effects of ethanol on adsorption on DVB-PDMS SPME fiber of: A) internals standards EMS, MIS, DES, and EIS; B) DMS, MeSOAc, DMDS, and EtSOAc

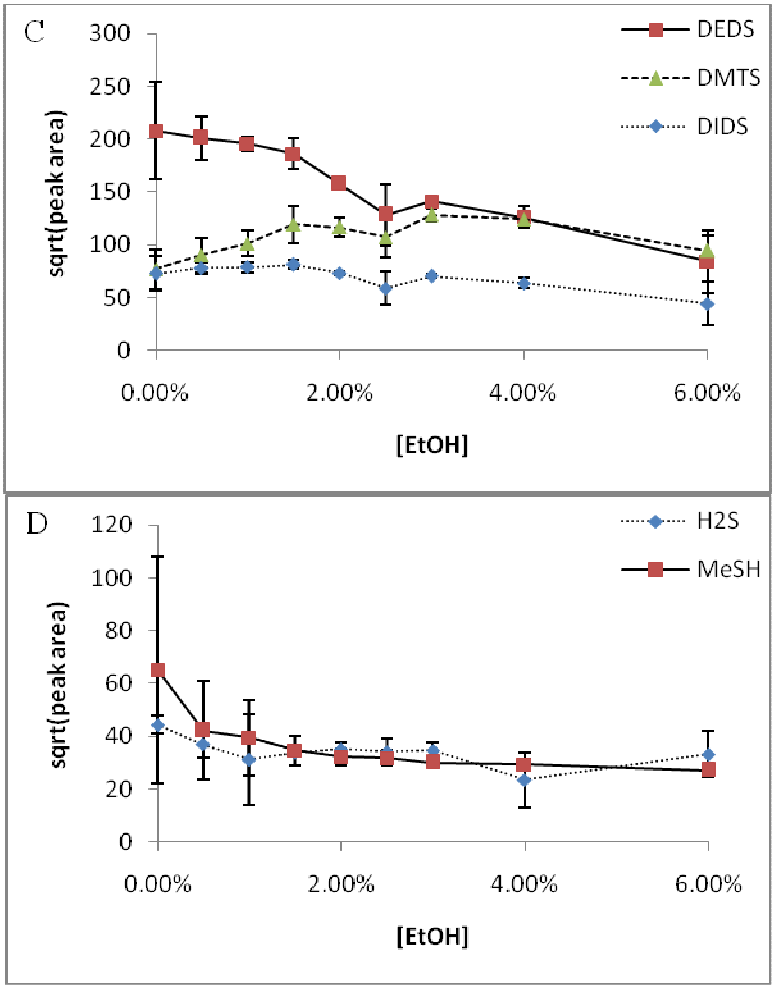


Figure 2.3 (continued): Effects of ethanol on adsorption on DVB-PDMS SPME fiber of: C) large compounds DMTS, DEDS, and DIDS (IS); D) highly-volatile compounds MeSH and H_2S

Traditionally, EMS and DIDS have been used as internal standards to measure smaller (DMS, thioacetates, etc.) compounds and larger (DEDS, DMTS) compounds respectively [28, 62], though other compounds have been used including hexylmercaptan, propylthioacetate [98], 4-methylthiobutanol [28], thiophene [72], etc. In addition to the traditional EMS and DIDS, this study used internal standards DES, MIS, and EIS. These sulfides were selected for the protective nature of their large substituents, in an effort to reduce outside influence on the central sulfur atom. Diisopropyl sulfide (DIS) was tried, but co-eluted with ethanol, causing an irregular peak due to quenching effects.

Results from analyte-to-internal-standard ratio tests on carboxen-PDMS fiber are shown in figure 2.4. Because the carboxen-PDMS fiber had the greatest sensitivity, and the ratios were at least as consistent as the other fibers, it was decided to only pursue a single fiber for analysis. The ratio results of other fibers are seen in the appendix. DMS is most closely matched by EMS, as indicated by a flat curve shape throughout ethanol-concentration increase. However, all other analytes show a large discrepancy compared to EMS. The data suggests that DMDS and the thioacetates may be better suited with DES or EIS; DEDS and DMTS seem to most closely follow DIDS. H_2S and MeSH, counter-intuitively, seem to follow DIDS, rather than EMS as might be expected from their size. The direct-analysis curves of H_2S, MeSH, and DIDS show a similar gradual decrease with rising ethanol concentration, suggesting a less extreme influence. This may be a function of molecular size and displacement on the SPME fiber—DIDS is a relatively large compound that could displace smaller compounds, and H_2S and MeSH are small enough to fit in the minute pores of the carboxen phase and avoid displacement. This could

decrease the effect of ethanol competition for both, as more mid-sized compounds may lack the ability to displace ethanol or remain on the fiber against larger compounds. Furthermore, between 0.0% and 1.0% ethanol, all ratios are unstable. This suggests a minimum of 1.0% ethanol is required for accuracy, above which the ratios become more consistent.

Similar effects were seen using DVB-Carboxen-PDMS and DVB-PDMS fibers (see Appendix). However, because sensitivity was greatest on the traditional carboxen-PDMS fiber, and ratio-analysis suggests accurate measurements within defined ethanol ranges, it was decided to pursue the single fiber for further analysis. Based on the apparent stabilization above 1% EtOH, the ideal wine sample would be diluted to 2mL wine:8mL saturated salt water. Wine commonly ranges between 12-15% ethanol, which, after dilution, is reduced to 2.4-3%. It is within this range that analyte:IS ratios are best maintained, especially in the case of H_2S and MeSH, in which a consistent correlation was not found across the entire ethanol range. Thus, in order to avoid strong ethanol matrix effects, internal standards EMS, EIS, and DIDS should be used on carboxen-PDMS fiber. DMS should be measured against EMS; DMDS, MeSOAc, and EtSOAc should be measured against EIS, and DEDS, DMTS, H_2S, and MeSH should be measured against DIDS.

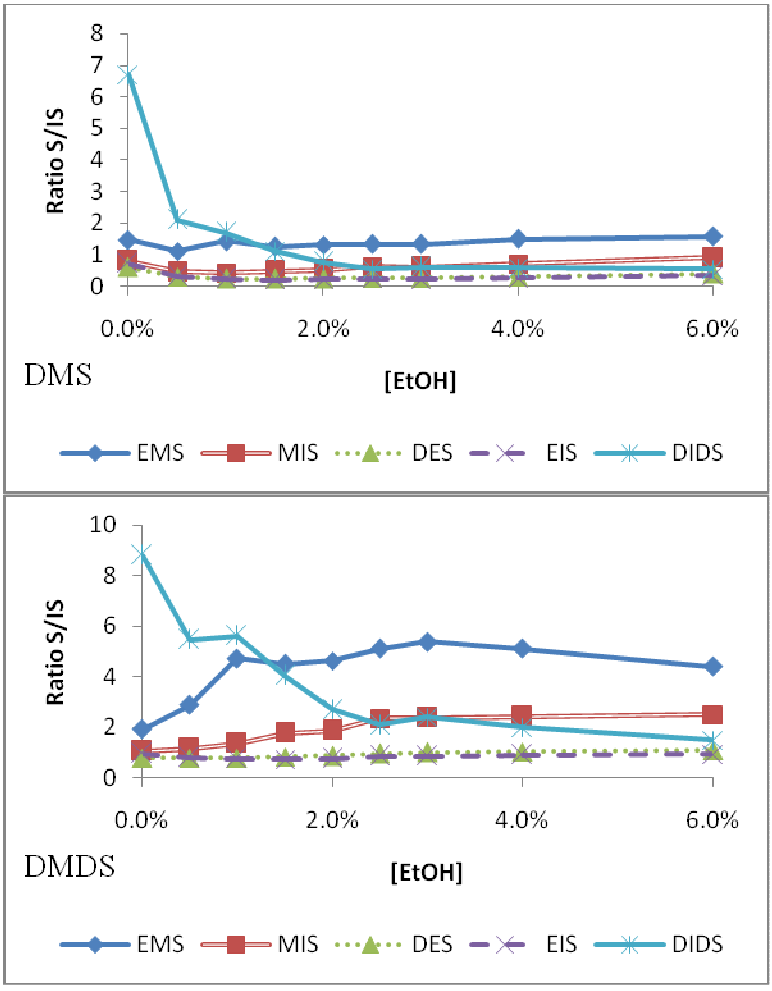


Figure 2.4: Analyte-to-internal-standard ratios (based on square roots of peak areas) to each of five internal standards of DMS, DMDS, MeSOAc, EtSOAc, DEDS, DMTS, H_2S, and MeSH

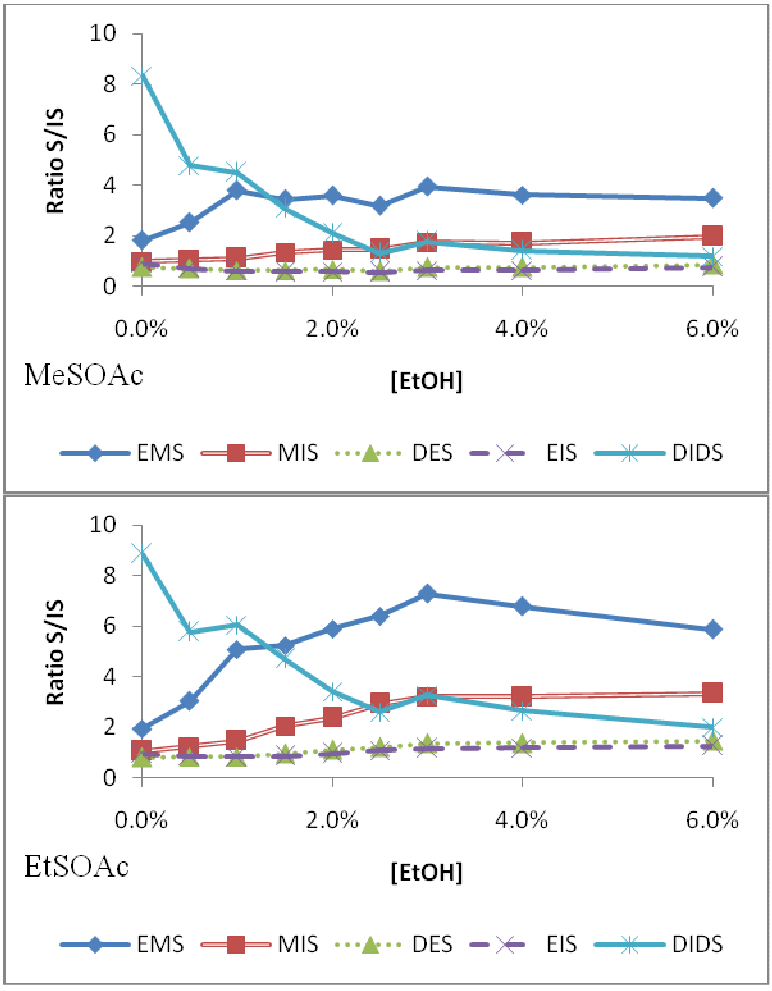


Figure 2.4 (continued): Analyte-to-internal-standard ratios (based on square roots of peak areas) to each of five internal standards of DMS, DMDS, MeSOAc, EtSOAc, DEDS, DMTS, H_2S, and MeSH

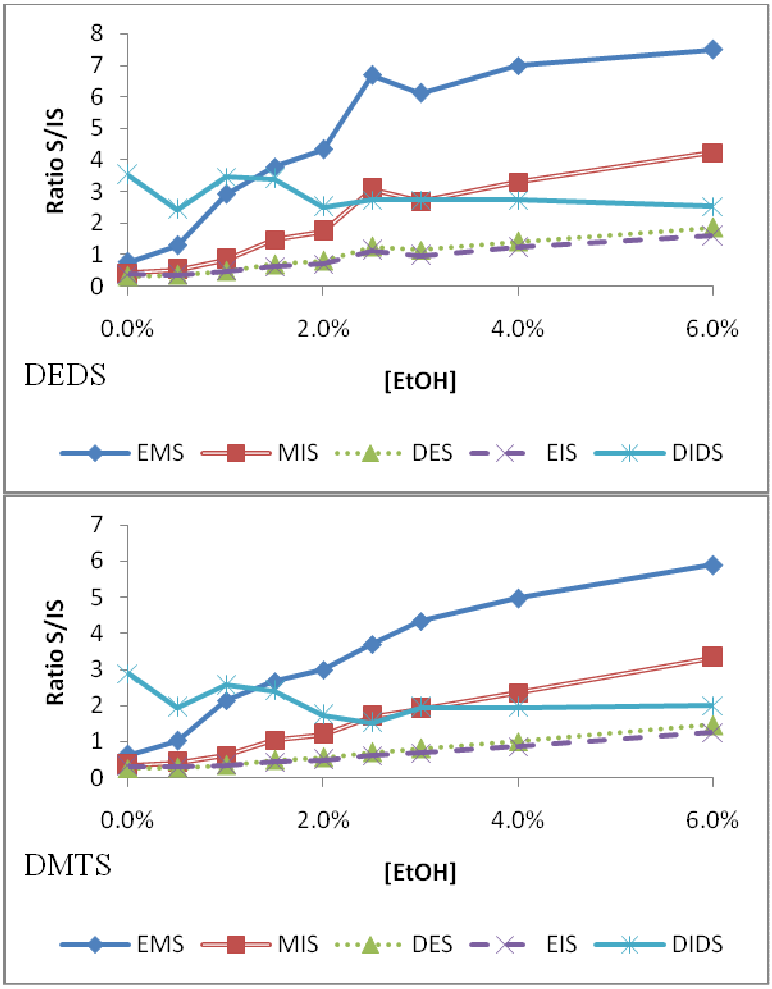


Figure 2.4 (continued): Analyte-to-internal-standard ratios (based on square roots of peak areas) to each of five internal standards of DMS, DMDS, MeSOAc, EtSOAc, DEDS, DMTS, H_2S, and MeSH

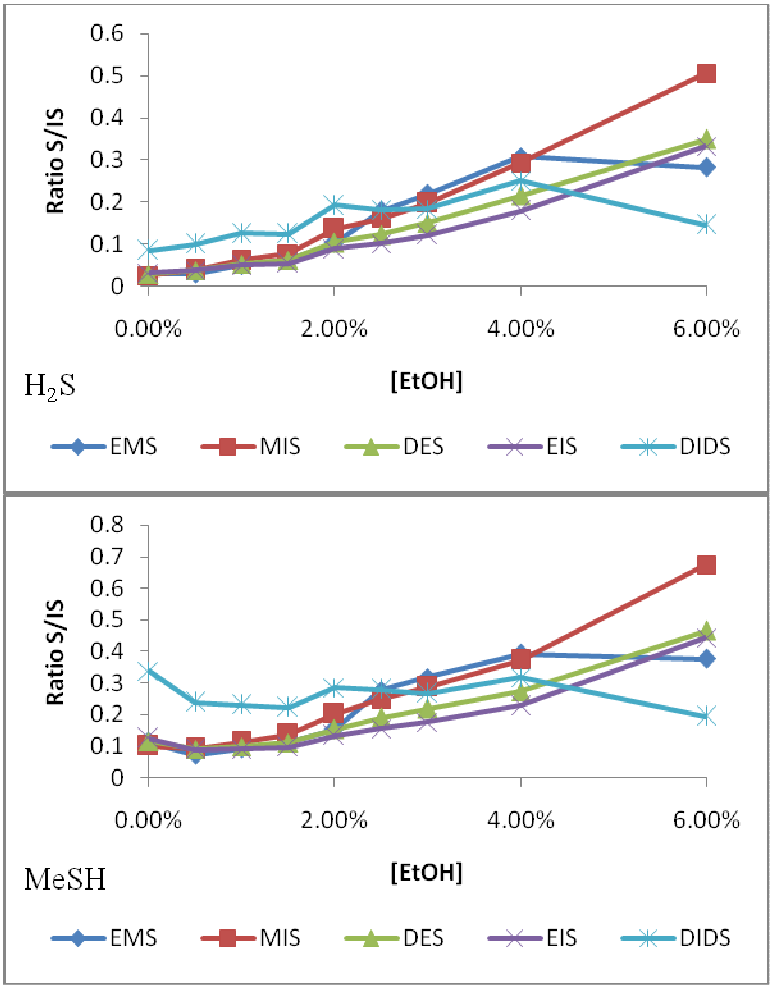


Figure 2.4 (continued): Analyte-to-internal-standard ratios (based on square roots of peak areas) to each of five internal standards of DMS, DMDS, MeSOAc, EtSOAc, DEDS, DMTS, H_2S, and MeSH

EFFECTS OF VOLATILE AND NON-VOLATILE WINE MATRIX ON VOLATILE-SULFUR ADSORPTION ON SPME FIBER

Peter M. Davis and Michael C. Qian

Abstract

The analysis of volatile sulfur compounds using headspace solid-phase microextraction (HS-SPME) is heavily influenced by matrix effects. The effects of a wine matrix, both non-volatile and volatile components (other than ethanol) were studied on the analysis of several common sulfur volatiles found in wine, including hydrogen sulfide (H_2S), methanethiol (MeSH), dimethyl sulfide (DMS), dimethyl disulfide (DMDS), dimethyl trisulfide (DMTS), diethyl disulfide (DEDS), methyl thioacetate (MeSOAc), and ethyl thioacetate (EtSOAc). Varying levels of devolatilized wine and common wine volatiles (acids, esters, alcohols) were added to synthetic wine samples to act as matrices. Sulfur standards were added and analyzed using gas chromatography with pulsed-flame photometric detection (GC-PFPD). Five internal standards were used to find best representatives of each compound despite matrix effects. Sensitivity remained stable with the addition of devolatilized wine, while addition of volatile components decreased sensitivity. DMS was found to be best measured against EMS; DMDS and the thioacetates were best measured against DES; H_2S, MeSH, DEDS, and DMTS were best measured against DIDS.

Introduction

The wine matrix is very complex, containing many different chemical classes and species. Pigments, phenolics, acids, volatile flavors, proteins, ethanol, etc. all play a role in the specific chemistry of a wine. The effects of the matrix can be seen on flavor analysis, acting as a protective source of antioxidants [100, 101], preventing flavor release [7], or reacting with desired analytes [102]. Several methods are used to concentrate volatiles and separate them from the matrix, including flavor trapping [4, 103], solid-phase extraction

[64, 95], and solid-phase microextraction (SPME). Because of its ease and speed of use, headspace-SPME (HS-SPME) has been adopted as the standard for flavor analysis [97]. However, this has not been without criticism [59, 60].

It has been shown that matrix effects have a significant effect on the extraction of sulfur volatiles using HS-SPME. Some attribute a loss of sensitivity to competition for limited adsorption space on the SPME fiber [63, 98], while others have seen the same effects using static headspace analysis [58, 99]. In the latter case, ethanol was suggested to act as a co-solvent for the compounds, limiting their ability to enter the headspace. Furthermore, not all sulfur volatiles are affected equally by matrix parameters [72].

This study aims to determine whether non-volatile or volatile components other than ethanol have an effect on the analysis of sulfur compounds using HS-SPME-gas chromatography with pulsed-flame photometric detection (GC-PFPD). Six devolatilized wines (DVWs) served as non-volatile matrix standards, and mixtures of most-prominent non-sulfur-containing volatiles in wine were used as volatile matrix standards, including acids, alcohols, and esters, based on reported ranges in wines [104].

Materials and Methods

Chemicals

Sodium sulfide, methanethiol (MeSH), dimethyl disulfide (DMDS), dimethyl trisulfide (DMTS), diisopropyl disulfide (DIDS), hexanoic acid, octanoic acid, phenethyl alcohol, 3-methyl-1-butanol, 2-methyl-1-propanol, ethyl acetate, 3-methyl-1-butyl acetate, ethyl hexanoate, and ethyl decanoate were from Sigma-Aldrich (St. Louis, MO, USA). Ethyl octanoate was from Eastman (Rochester, NY, USA). Methyl thioacetate (MeSOAc), ethyl thioacetate (EtSOAc), and diethyl sulfide (DES) were from Alfa-Aesar (Ward

Hill, MA, USA). Ethyl methyl sulfide (EMS), dimethyl sulfide (DMS), diethyl disulfide (DEDS), methyl isopropyl sulfide (MIS), and ethyl isopropyl sulfide (EIS) were from TCI America (Portland, OR, USA). Methanol was from EMD Chemicals Inc. (Gibbstown, NJ, USA), l-tartaric acid from J.T. Baker (Phillipsburg, NJ, USA), and ethanol was from Koptec (King of Prussia, PA, USA).

Sample Preparation

Standards

Hydrogen sulfide standards were prepared using equivalents of sodium sulfide (Na_2S) dissolved in distilled water, and further diluted with cold (-15ºC) methanol. MeSH standards were prepared by bubbling the pure gas over cold methanol and recording gained mass. All other standards were prepared by dilution with cold methanol. A standard mixture (mix 1) was prepared containing DMS (3000µg/L), MeSOAc (1285.5µg/L), DMDS (218.28µg/L), EtSOAc (564µg/L), DEDS (55.5µg/L), and DMTS (46.76µg/L). Because MeSH readily oxidizes to DMDS, and the higher affinity for DMDS on the SPME fiber causes much greater peak responses, the two compounds were not analyzed simultaneously. A separate mixture (mix 2) was thus prepared containing MeSH (36.9mg/L) and H_2S (31.25µg/L). Finally, a mixture of internal standards (IS mix) was prepared containing EMS (5mg/L), DES (1mg/L), MIS (1.5mg/L), EIS (1mg/L), and DIDS (25.9µg/L).

Volatile-matrix Standards

Four separate sets of volatile-matrix standards were prepared; these consisted of acids (acetic, hexanoic, octanoic, decanoic), alcohols (2-methyl-1-propanol, 3-methyl-1-butanol, phenethyl alcohol), esters (ethyl acetate, 3-

methyl-1-butyl acetate, ethyl hexanoate, ethyl octanoate, ethyl decanoate) and a total mixture of all three. Each set was prepared by diluting the respective compounds in cold (4ºC) ethanol. Final concentrations of each compound in the acid and alcohol mixtures (after added to synthetic wine to reflect base wine concentration) were 1, 2, 3, 4, 5, and 6mg/L. Final concentrations of each compound in the ester mixture were 0.1, 0.25, 0.5, 1, 2, and 3mg/L. Final concentrations of each compound in the total mixture of acids, alcohols, and esters, were the same as in their respective mixtures. The cumulative concentration of compounds in the highest level (level 6) of the total mixture consisted of four acids each at 6mg/L, three alcohols each at 6mg/L, and five esters each at 3mg/L, thus 57mg/L total.

Non-volatile matrix

Three wines were supplied by E&J Gallo Winery (Modesto, CA, USA) to be devolatilized, consisting of: Louis Martini Cabernet Sauvignon (2009), Gallo Family Vineyards Pinot Grigio (blend), and Dancing Bull Sauvignon Blanc (2009). A pinot noir (2007) and chardonnay (2007) from Argyle Winery (Dundee, OR, USA) and a merlot (2004) from Hogue Cellars (Prosser, WA, USA) were also used. Wines were devolatilized as follows: 300mL of wine was boiled using a rotary evaporator (Büchi, Switzerland) under vacuum at 40ºC and 85rpm. Each wine was boiled until 40% remained (120mL), then distilled water was added back to original concentration. This ensured all volatile compounds had been evaporated, including ethanol.

Samples

Samples were prepared in 20mL deactivated screw-cap glass vials with Teflon-faced silicone septa. Devolatilized wine samples were prepared using

varying levels of DVW, consisting of 0, 10, 15, 20, 25, 30, and 40% wine matrix. Ethanol (0.3mL) and saturated salt water were added to reach a final volume of 10mL, and final ethanol concentration of 3%. Vials were flushed gently with argon under low flow rate (barely disturbing the surface of the sample liquid) to avoid turbulence. All samples received 20µL of standard mixture (mix 1 or 2) and 10µL of IS mix (30µL of methanolic solutions added in total). In the case of mix 2, all standards were introduced via syringe through the sample-vial septum to avoid oxygen contact. All standards were stored in the freezer (-15ºC).

Volatile-matrix samples consisted of 2mL synthetic wine (3.6g/L tartaric acid) at 15% ethanol. Salt water was added to reach a final volume of 10mL and final ethanol concentration of 3%. Volatile compound sets (i.e. acids, alcohols, esters, or total) were added at 20µL at each level, to reach final concentrations listed. In an effort to consolidate sulfur analysis, a combination of mix 1 and mix 2 was prepared containing all sulfur standards. However, because of the oxidation of MeSH to DMDS, DMDS was not measured. To each sample 20µL of sulfur-standards mix and 10µL of internal standards mix was added, reaching a final addition of 50µL standards. All standards were added via syringe to avoid oxygen intake.

SPME Conditions

The SPME fiber used was an 85µm Carboxen-PDMS (Supelco, Bellafonte, PA). The samples were equilibrated at 30ºC for 5 minutes and the extraction took place for 20 min with agitation at 250rpm. Injection temperature was 300ºC. Samples were analyzed in triplicate.

GC-PFPD

Samples were run on a Varian CP-3800 gas chromatograph equipped with a pulsed-flame photometric detector (PFPD; Varian, Walnut Creek, CA). A DB-FFAP column (30m x 0.32mm x 1µm, Agilent, Palo Alto, CA) was used for separation. A temperature program was used for the GC oven: 35ºC for 3min, ramped to 150ºC at 10ºC/min, held 5min, ramped to 220ºC at 20ºC/min, held 3min. Nitrogen was used as carrier gas at 2mL/min flow rate. Detector temperature was 300ºC with 14mL/min hydrogen, 17mL/min air 1, and 10mL/min air 2. The PFPD was operating in sulfur mode, with 6ms gate delay and 20ms gate width. Data analysis relied on square roots of peak areas.

Results and Discussion

Non-volatile Matrix Effects

Results of DVW effects on sulfur extraction from chardonnay wine are shown in figure 3.1. Though a very gradual decrease can be seen in all compounds, there is very little effect seen in the chardonnay wine. Similar curves are seen for all other wine matrices (figure 3.2). The slight decrease as DVW content rises is likely due to a decrease in salt in the system, as 40% DVW reduces salt water content to less than 6mL.

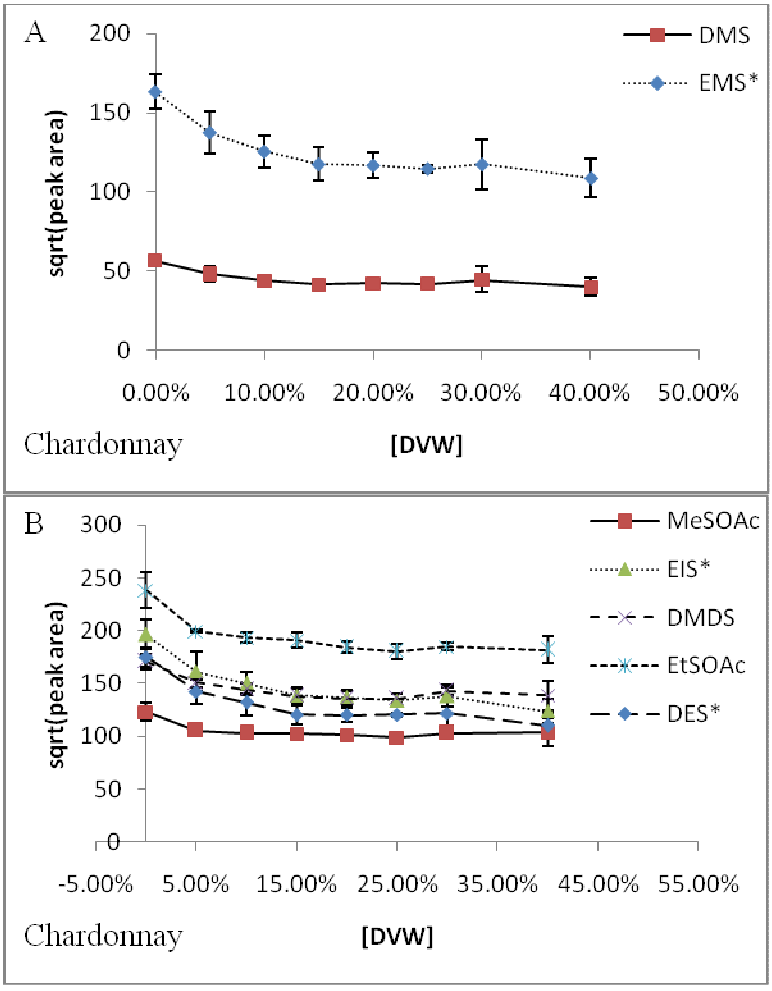


Figure 3.1: Affects of non-volatile wine matrix using chardonnay devolatilized wine (DVW) on HS-SPME analysis of: A) DMS and EMS (IS), B)MeSOAc, DMDS, EtSOAc, DES (IS), and EIS (IS)

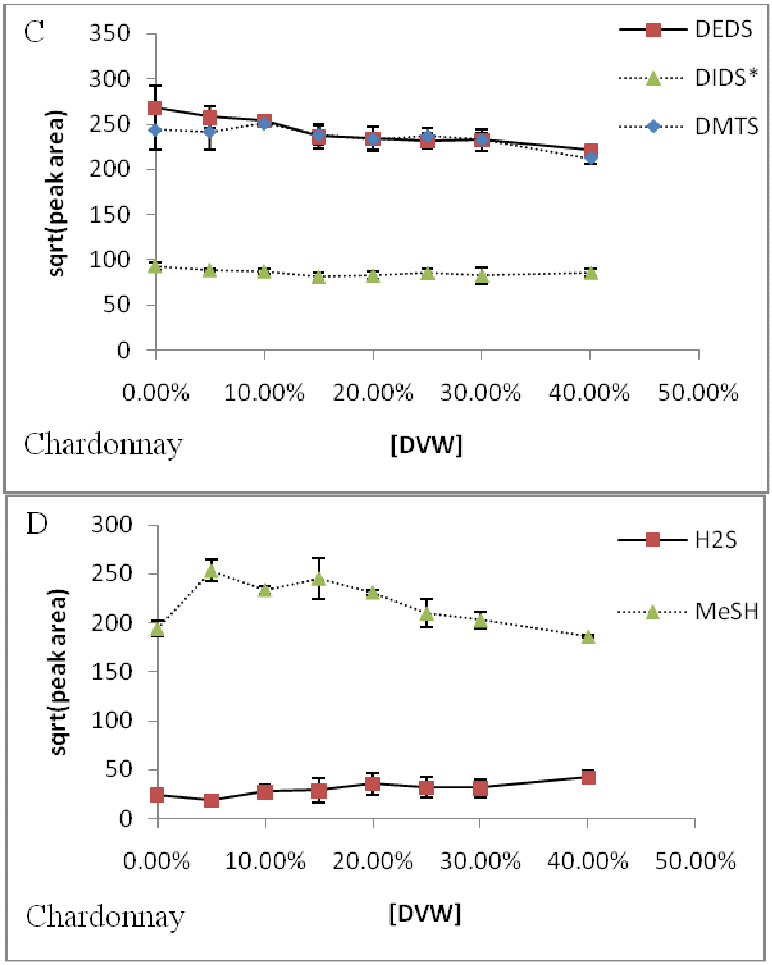


Figure 3.1 (continued): Affects of non-volatile wine matrix using chardonnay devolatilized wine (DVW) on HS-SPME analysis of: C) DEDS, DMTS, and DIDS (IS), D) H_2S, and MeSH

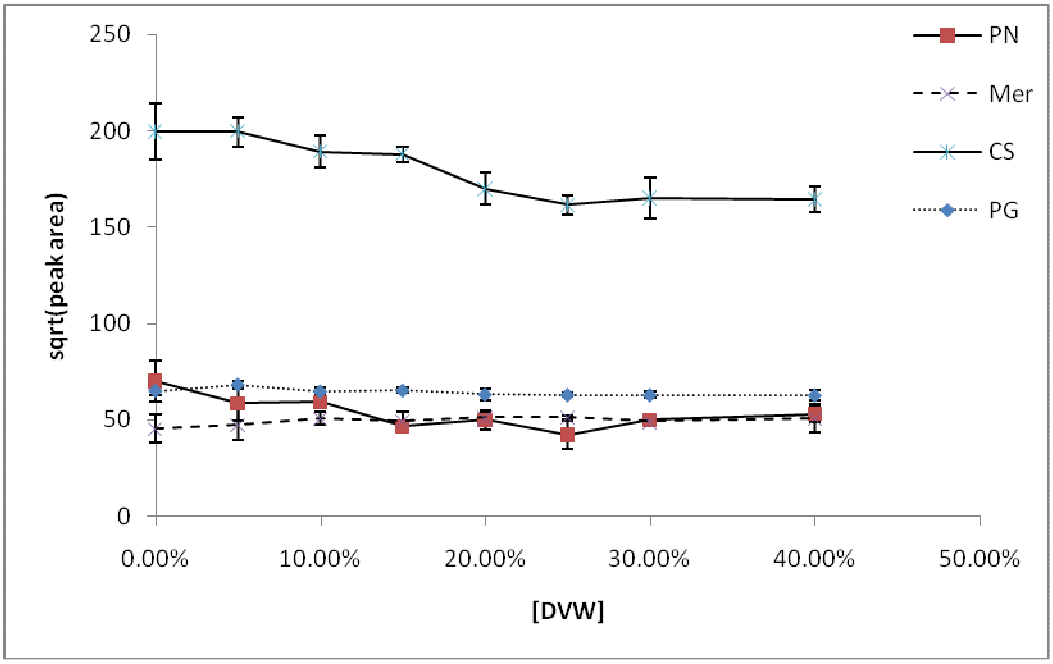


Figure 3.2: Effects of various DVW matrices on DMS extraction: PN=Pinot Noir, Mer=Merlot, CS=Cabernet Sauvignon, PG=Pinot Grigio

More important are the ratios of analytes to internal standards (figure 3.3), which gauge how closely the intended internal standard resembles the analyte in question. DMS, DMDS, MeSOAc, EtSOAc, DEDS, and DMTS all show very consistent ratios as DVW concentration increases. DMS closely matches EMS; DMDS, MeSOAc, and EtSOAc all closely follow EIS, DES, and EMS, though ethanol-effect studies have suggested EIS is ideal. DEDS and DMTS are well-represented by DIDS.

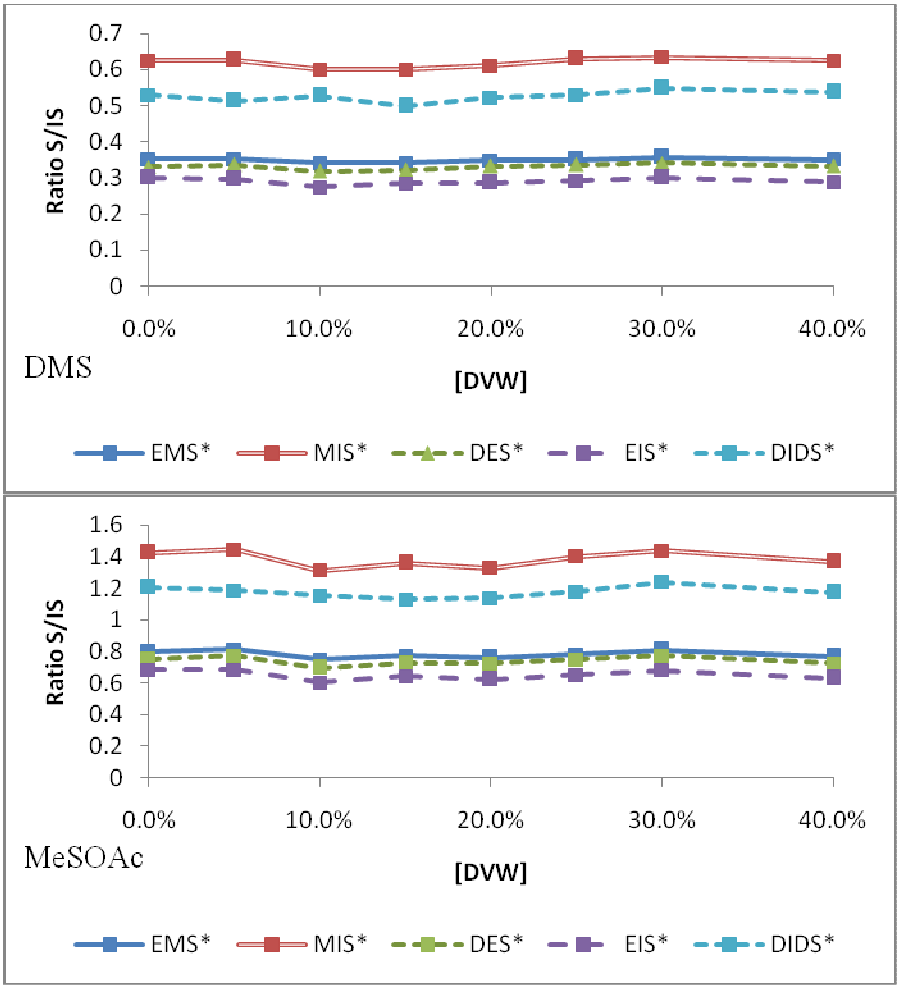


Figure 3.3: Analyte-to-internal-standard ratios to all five internal standards in merlot DVW of DMS, MeSOAc, DMDS, EtSOAc, DEDS, and DMTS

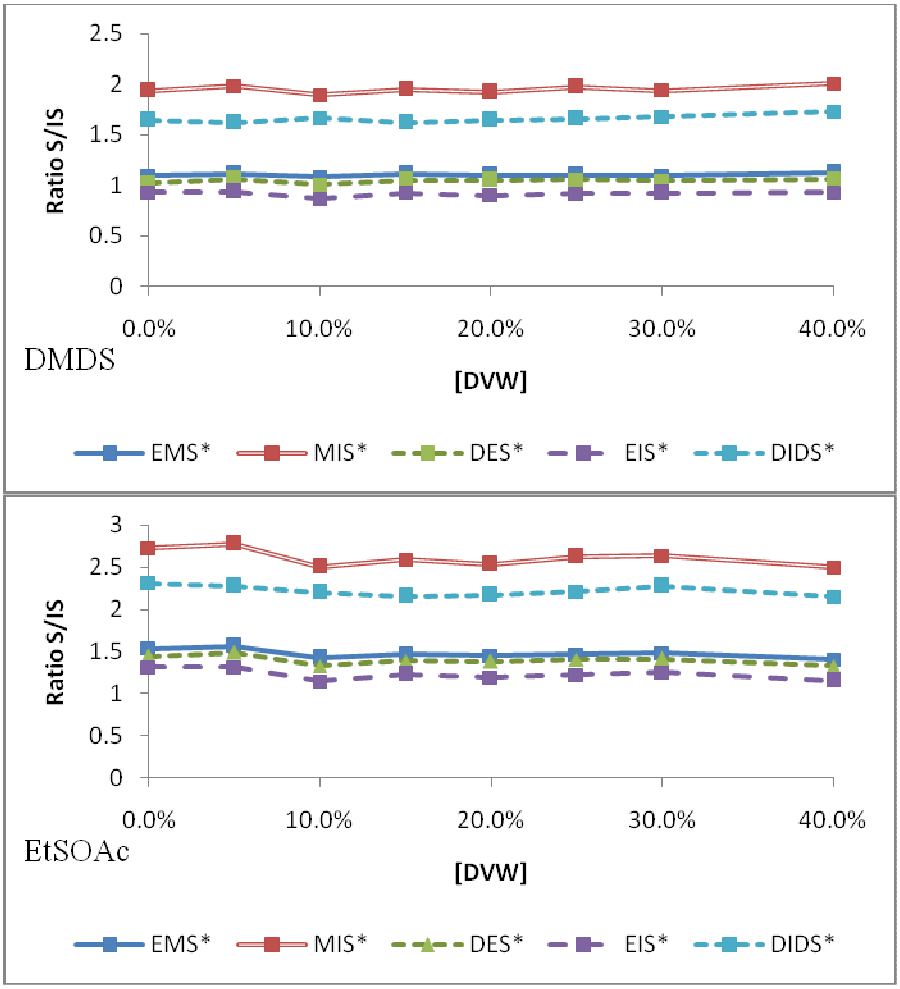


Figure 3.3 (continued): Analyte-to-internal-standard ratios to all five internal standards in merlot wine of DMS, MeSOAc, DMDS, EtSOAc, DEDS, and DMTS

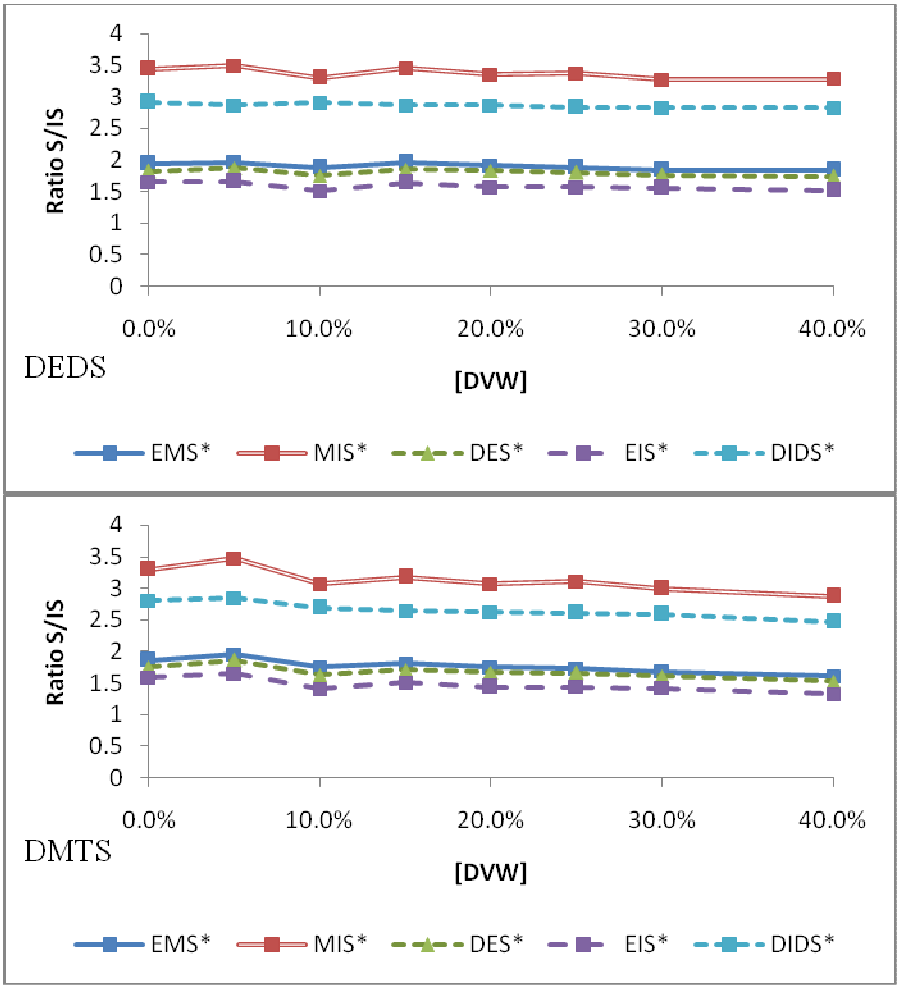


Figure 3.3 (continued): Analyte-to-internal-standard ratios to all five internal standards in merlot wine of DMS, MeSOAc, DMDS, EtSOAc, DEDS, and DMTS

Volatile-matrix Effects

The analyses of volatile sulfur compounds with varying levels of other (non-sulfur) volatiles are shown in figure 3.4. Data is arranged by volatile-matrix level, ranging from 0 (no additional volatiles added) to 6. These correlate to the aforementioned concentrations of each compound in each set (acids, esters, alcohols). Analysis of the total mixture was performed foremost,

in order to gauge effects; the total mixture most closely reflects that of a wine, which would not be completely deficient in one category. Thus, within a wine, the volatiles would have a cumulative effect as measured. Results from this total mixture best exemplify the effects of other volatile constituents on SPME adsorption of sulfur compounds.

As seen with ethanol, a strong decrease in the adsorption of volatile sulfur compounds is seen with increasing volatile-profile concentration. This suggests a competitive mechanism, as the volatile matrix components will fill the headspace and adhere to the fiber. The concentrations of each volatile added are insufficient to act as co-solvents as ethanol might, though may affect the equilibrium of volatiles in the headspace as more compounds become present [58, 99].

The analyte-to-internal-standard ratios (figure 3.5) showed high variation. DMS still closely follows EMS. In ethanol studies, MeSOAc and EtSOAc both resemble EIS and DES, suggesting they might be accurate internal standards. However, the volatile-matrix data suggests that EIS loses its similarity at higher concentrations of volatiles. While EMS seems to match closely with both, ethanol studies showed it did not function well with varied ethanol content. Thus, DES is the internal standard of choice for the thioacetates. DEDS and DMTS, similar to the thioacetates, show good correlation with EMS. However, ethanol studies also suggested that DIDS was the only viable internal standard. H_2S and MeSH are not well-represented by any of the internal standards, though seem to correlate with EMS and DIDS. EMS was not found to correlate well with shifting alcohol contents, however, so DIDS remains the most viable internal standard for both.

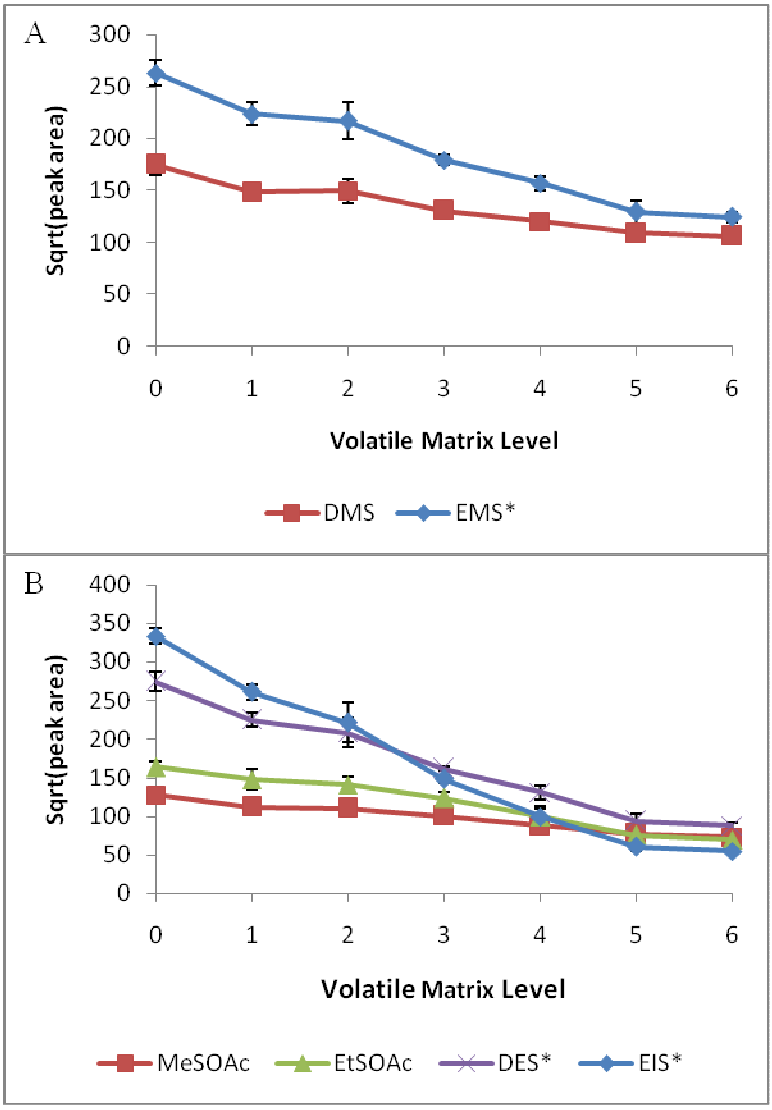


Figure 3.4: Effects of volatile mixture of acids, alcohols, and esters (across reported ranges in wine) on SPME adsorption of: A) DMS and EMS (IS), B) MeSOAc, EtSOAc, DES (IS), EIS (IS)

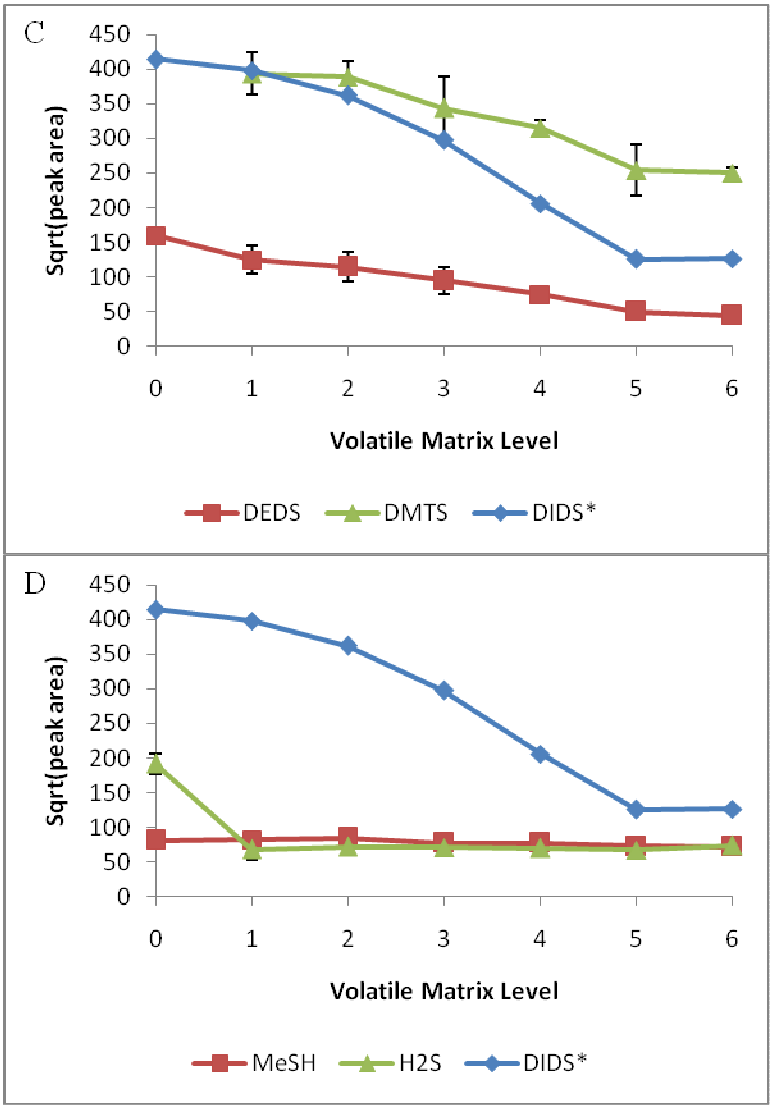


Figure 3.4 (continued): Effects of volatile mixture of acids, alcohols, and esters (across reported ranges in wine) on SPME adsorption of: C) DEDS, DMTS, and DIDS (IS), D) MeSH, H_2S, and DIDS (IS)

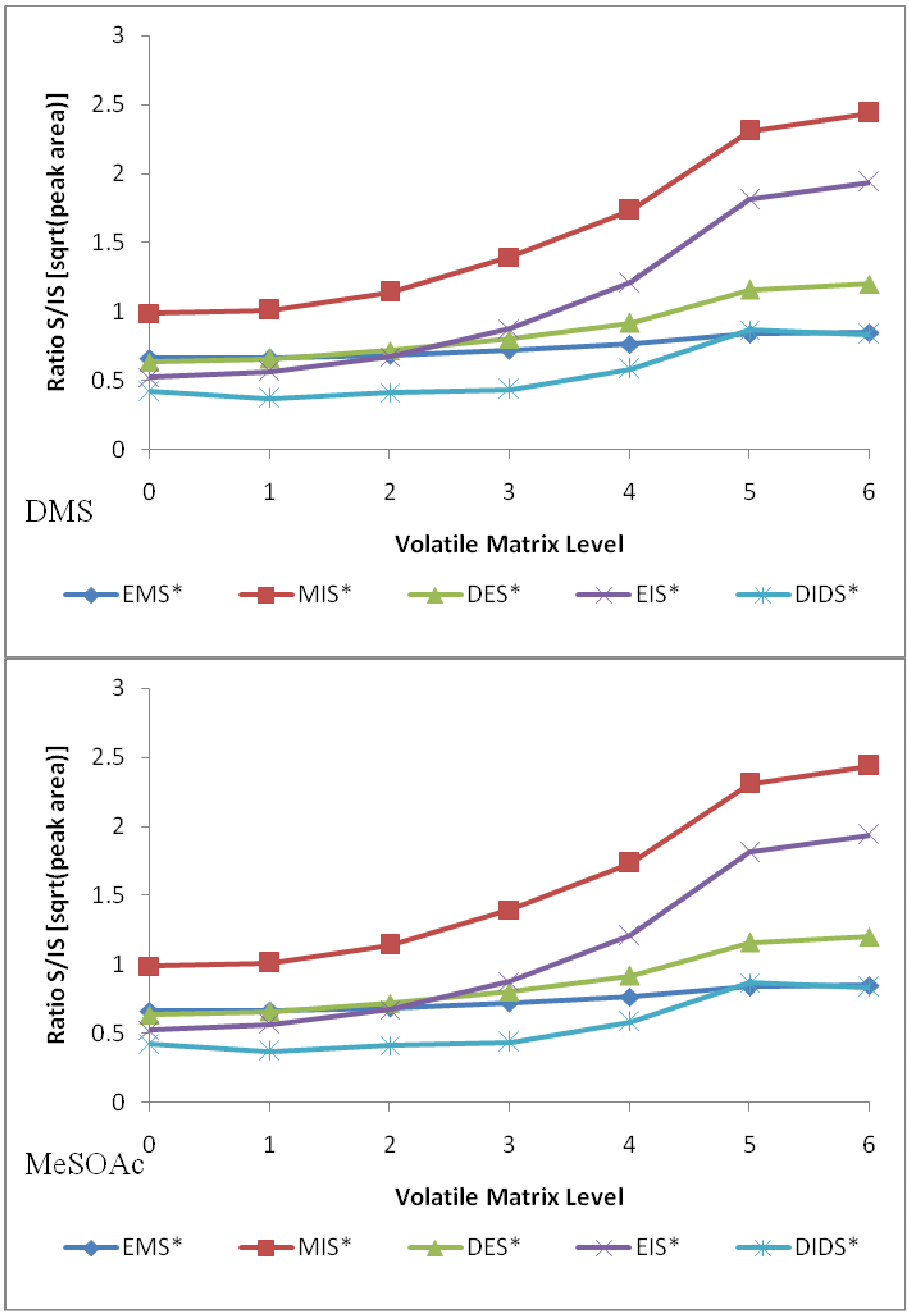


Figure 3.5: Effect of volailte matrix on analyte-to-internal-standard ratios against all five internal standards of DMS, MeSOAc, EtSOAc, DEDS, DMTS, H_2S, and MeSH

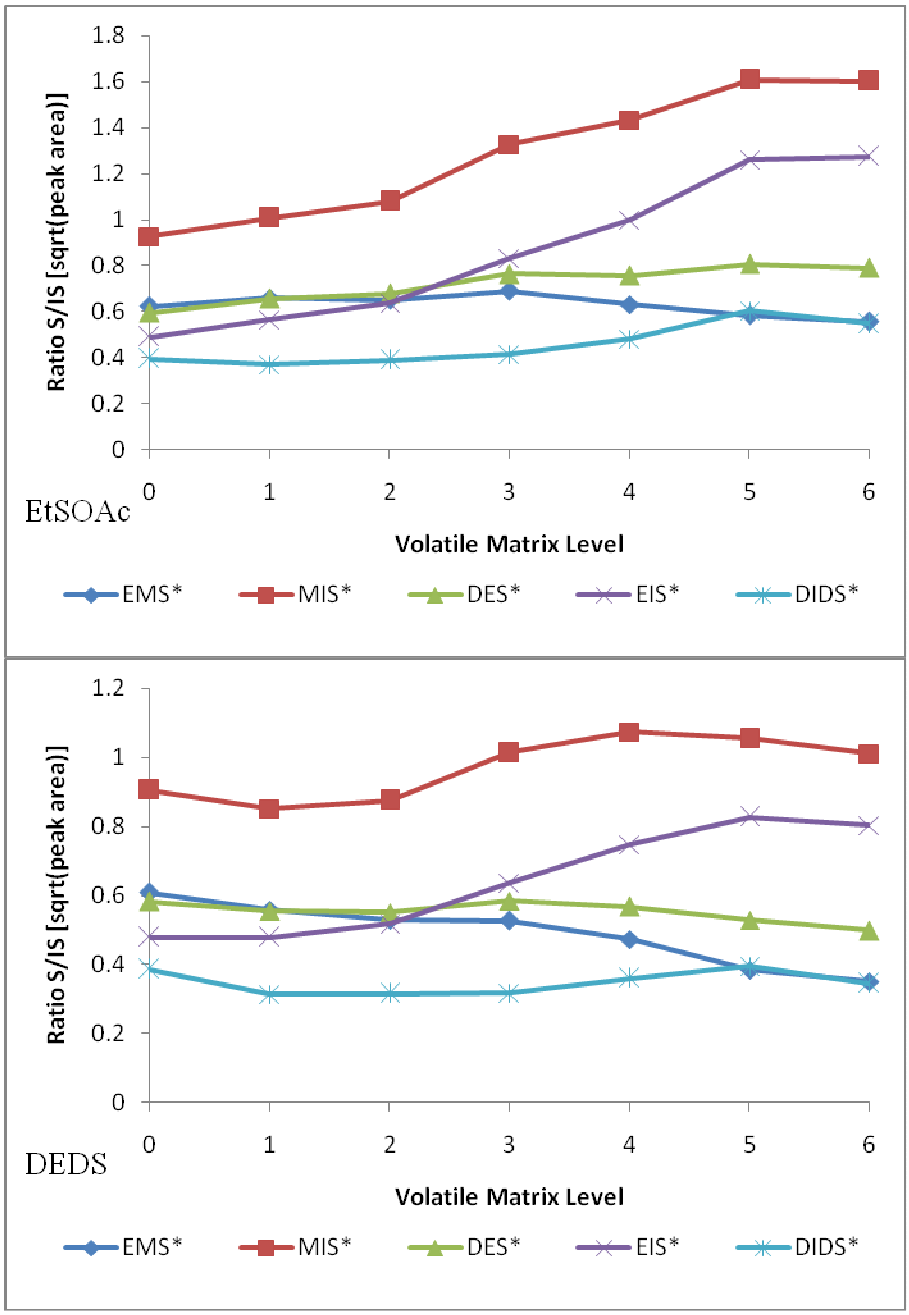


Figure 3.5 (continued): Analyte-to-internal-standard ratios against all five internal standards of DMS, MeSOAc, EtSOAc, DEDS, DMTS, H_2S, and MeSH

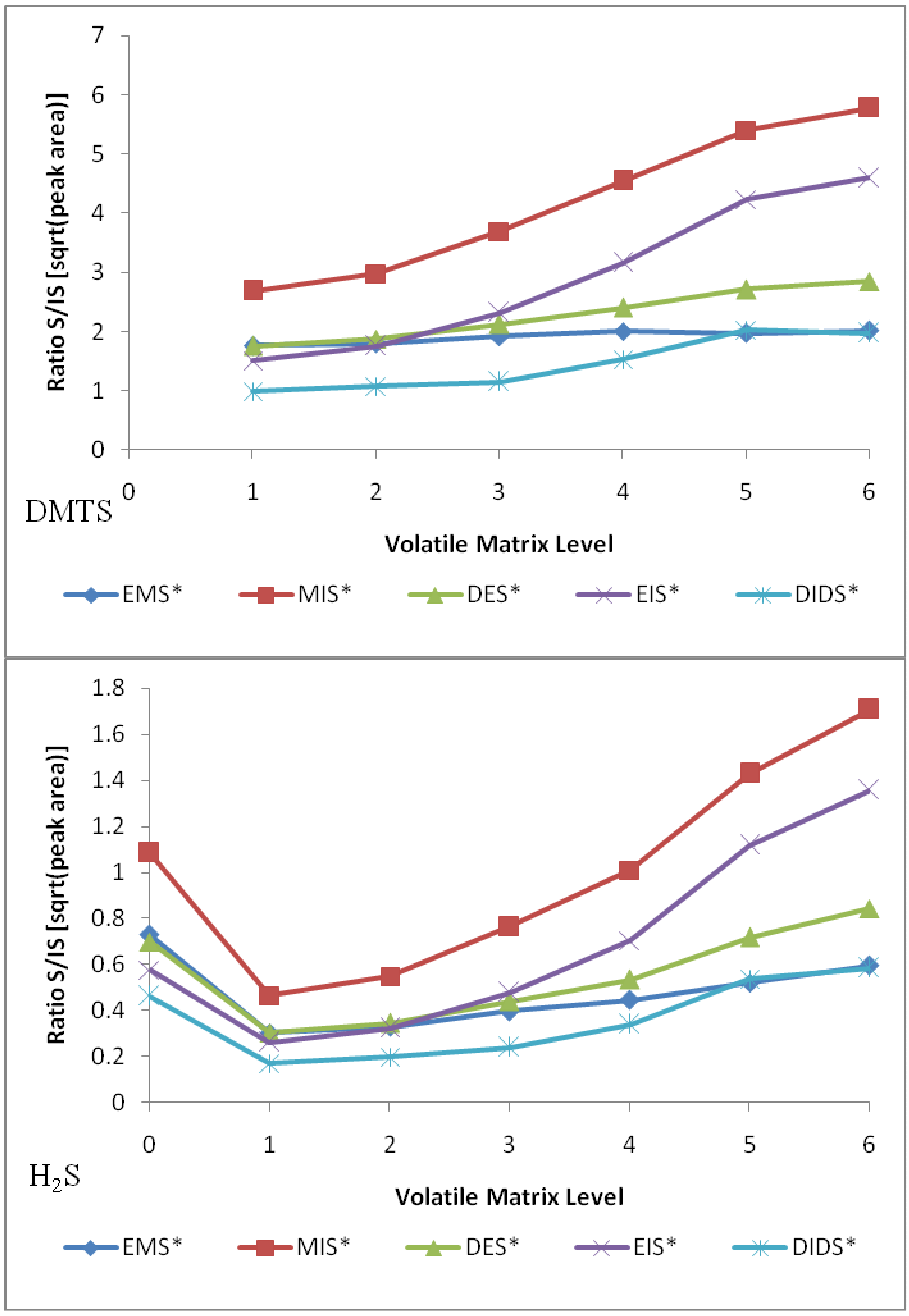


Figure 3.5 (continued): Analyte-to-internal-standard ratios against all five internal standards of DMS, MeSOAc, EtSOAc, DEDS, DMTS, H_2S, and MeSH

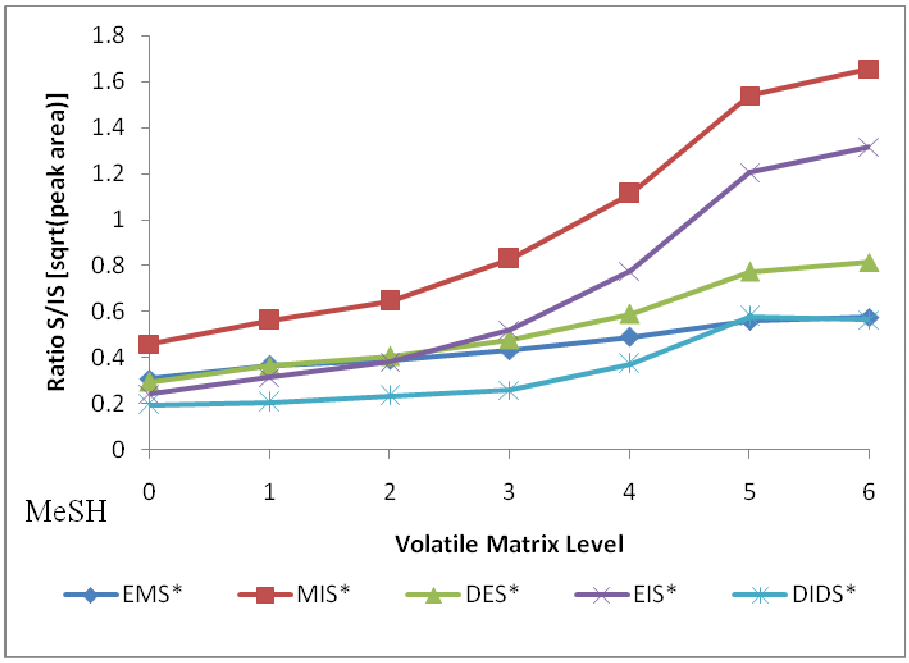


Figure 3.5 (continued): Analyte-to-internal-standard ratios against all five internal standards of DMS, MeSOAc, EtSOAc, DEDS, DMTS, H_2S, and MeSH

The analysis of sulfur compounds using HS-SPME is heavily influenced by the presence of other volatiles. Little effect is seen by non-volatile matrix components. Based on the results of both studies, ideal internal standards to compensate for variation of these parameters in multiple wines are EMS (for DMS), DES (for DMDS, MeSOAc, and EtSOAc), and DIDS (for H_2S, MeSH, DEDS, and DMTS).

QUANTIFICATION OF VOLATILE SULFUR COMPOUNDS IN MULTIPLE WINES USING HS-SPME-GCPFPD

Peter M. Davis and Michael C. Qian

Abstract

A method for analyzing volatile sulfur compounds in wine using headspace solid-phase microextraction (HS-SPME) with gas chromatography and pulsed-flame photometric detection (GC-PFPD) was developed. The method was designed to compensate for matrix effects, based on appropriate internal standards for each compound. Using this method, calibration curves for dimethyl sulfide (DMS), dimethyl disulfide (DMDS), methyl thioacetate (MeSOAc), ethyl thioacetate (EtSOAc), diethyl disulfide (DEDS), and dimethyl trisulfide were calibrated with good linearity (R^2>0.99); H_2S and MeSH also showed R^2>0.97. The method was used to quantify sulfur volatiles in 21 California wines.

Introduction

Volatile sulfur compounds pose a problem for winemakers as they exhibit strong off-odors of onion, garlic, cabbage, cheese, and rubber [22]. Analysis of these compounds poses a problem, however, because of their high volatility and low concentrations [60, 69]. Furthermore, analysis in a wine matrix creates complications, as the matrix, including ethanol, can affect sensitivity [58, 98]. Different sulfur volatiles are not necessarily affected in the same way by the matrix [63]. This study aims to analyze volatile sulfur compounds in 21 different wines using a method created to compensate for the matrix effect by selecting internal standards that behave most similarly to target analytes. Analysis used headspace solid-phase microextraction (HS-SPME) gas chromatography with pulsed-flame photometric detection (GC-PFPD).

Materials and Methods

Chemicals

Sodium sulfide, methanethiol (MeSH), dimethyl disulfide (DMDS), dimethyl trisulfide (DMTS), diisopropyl disulfide (DIDS), and acetaldehyde were from Sigma-Aldrich (St. Louis, MO, USA). Methyl thioacetate (MeSOAc), ethyl thioacetate (EtSOAc), and diethyl sulfide (DES) were from Alfa-Aesar (Ward Hill, MA, USA). Ethyl methyl sulfide (EMS), dimethyl sulfide (DMS), and diethyl disulfide (DEDS) were from TCI America (Portland, OR, USA). Methanol was from EMD Chemicals Inc. (Gibbstown, NJ, USA), l-tartaric acid from J.T. Baker (Phillipsburg, NJ, USA), and ethanol was from Koptec (King of Prussia, PA, USA).

Wines

Wines were provided by E&J Gallo Winery. A total of 21 California wines were analyzed, consisting of 13 red and 8 white, 9 different varietals, and 2 blends. Wine samples were prepared by diluting 2mL wine to 10mL with saturated salt water and adding 10µL internal standard mix and 5µL of 20mg/L acetaldehyde to counteract SO_2 [28].

Calibration of Sulfur Compounds

Hydrogen sulfide standards were prepared using equivalents of sodium sulfide (Na_2S) dissolved in distilled water, and further diluted with cold (-15ºC) methanol. MeSH standards were prepared by bubbling the pure gas over cold methanol and recording gained mass. All other standards were prepared by dilution with cold methanol. Because MeSH readily oxidizes to DMDS, and the higher affinity for DMDS on the SPME fiber causes much greater peak

responses, the two compounds were not calibrated simultaneously. Thus, MeSH was analyzed individually, and the remaining compounds were combined in a mixture for calibration. A mixture containing EMS (5mg/L), DES (1mg/L), and DIDS (25.9µg/L) was used for internal standards. Calibration samples consisted of 2mL synthetic wine (3.6g/L tartaric acid) diluted to 10mL with saturated salt water and ethanol, for a final ethanol content of 3%. Vials were flushed with argon and internal standards mixture (10µL) and analyte calibration levels (20µL) were added through the septum.

SPME

The SPME fiber used was an 85µm Carboxen-PDMS (Supelco, Bellafonte, PA). The samples were equilibrated at 30ºC for 5 minutes and the extraction took place for 20 min with agitation at 250rpm. Injection temperature was 300ºC.

GC-PFPD

Samples were run on a Varian CP-3800 gas chromatograph equipped with a pulsed-flame photometric detector (PFPD; Varian, Walnut Creek, CA). A DB-FFAP column (30m x 0.32mm x 1µm, Agilent, Palo Alto, CA) was used for separation. A temperature program was used for the GC oven: 35ºC for 3min, ramped to 150ºC at 10ºC/min, held 5min, ramped to 220ºC at 20ºC/min, held 3min. Nitrogen was used as carrier gas at 2mL/min flow rate. Detector temperature was 300ºC with 14mL/min hydrogen, 17mL/min air 1, and 10mL/min air 2. The PFPD was operating in sulfur mode, with 6ms gate delay and 20ms gate width. Data analysis relied on square roots of peak areas.

Results and Discussion

A typical chromatogram for wine analysis is shown in figure 4.1. This chromatogram represents a cabernet sauvignon wine. Calibration curves are shown in figure 4.2. Curves were constructed to represent a range of concentrations near the odor threshold, as well as potential levels in wines (table 1.1). Good linearity was seen for all curves, with R^2 values greater than 0.99 for DMS, DMDS, MeSOAc, EtSOAc, DEDS, and DMTS. Highly-volatile compounds H_2S and MeSH achieved R^2 values greater than 0.97.

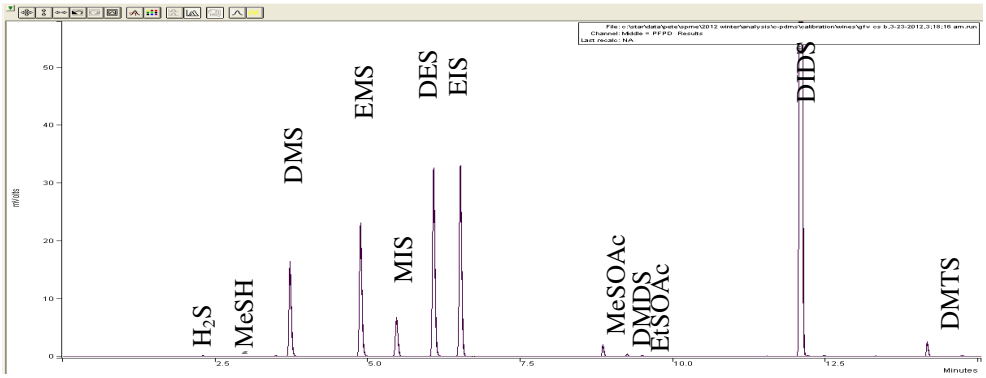


Figure 4.1: Representative GC-PFPD chromatogram of wine (cabernet sauvignon)

The results of the analysis of 21 California wines are seen in table 4.1. Traces of most compounds were found in all samples. Many wines had quantifiable levels of each sulfur compound, save DEDS, which was rarely found in greater than trace amounts. H_2S was found frequently in trace amounts, though it may still be present in perceivable concentrations. Due to the broad range reported for its odor threshold value [29, 30], it may be perceived at levels beneath its limit of detectability. White varietals like

chardonnay exhibit greater levels of H_2S and MeSH than reds. DMS was found in slightly higher concentrations in red varietals, particularly cabernet sauvignon and merlot. DMS and DMTS were the only compounds found consistently in all wines. Levels for DMS suggest little impact on the flavor of the wines, as concentrations slightly above the odor threshold are said to impart a beneficial fruity aroma [25].

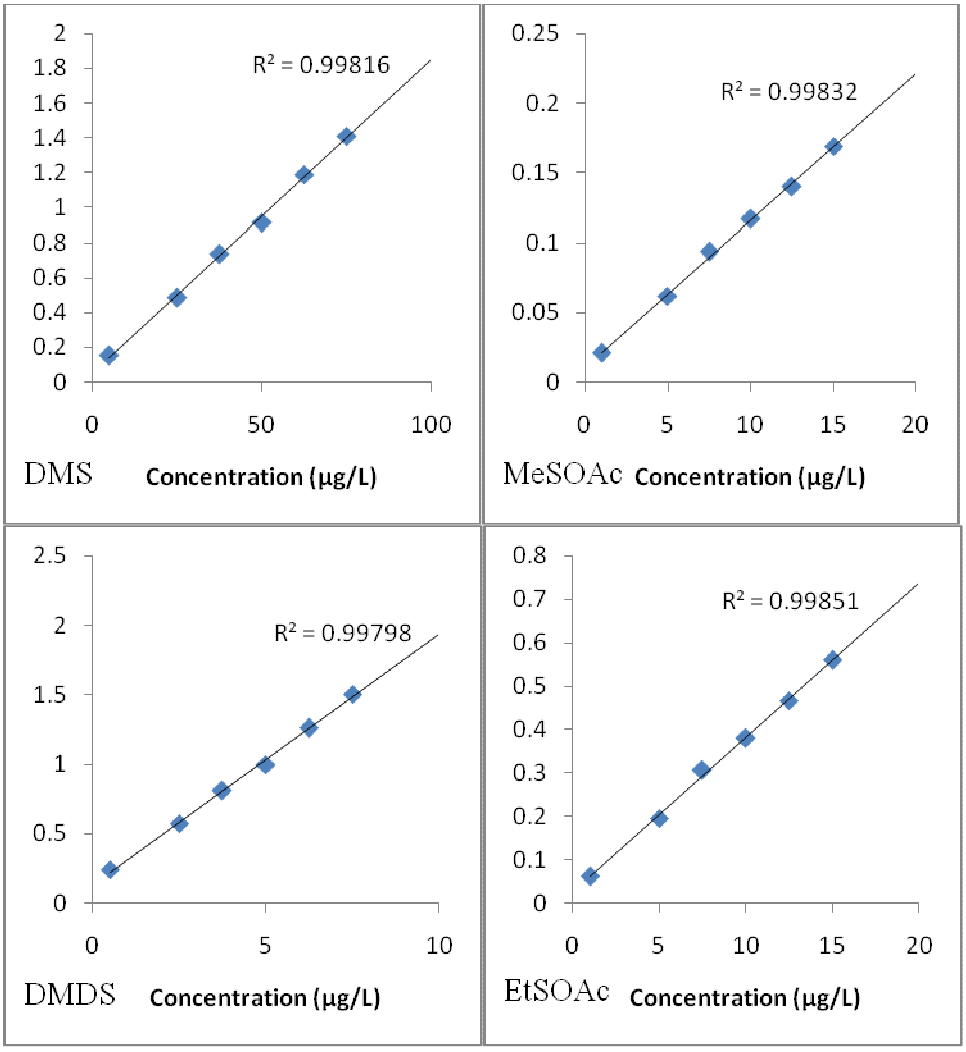


Figure 4.2: Calibrations curves for sulfur standards; y-axis = ratio of S:IS response (sqrt(peak area)); DMS (IS=EMS); DMDS, MeSOAc, EtSOAc (IS=DES); H_2S, MeSH, DEDS, and DMTS (IS=DIDS)

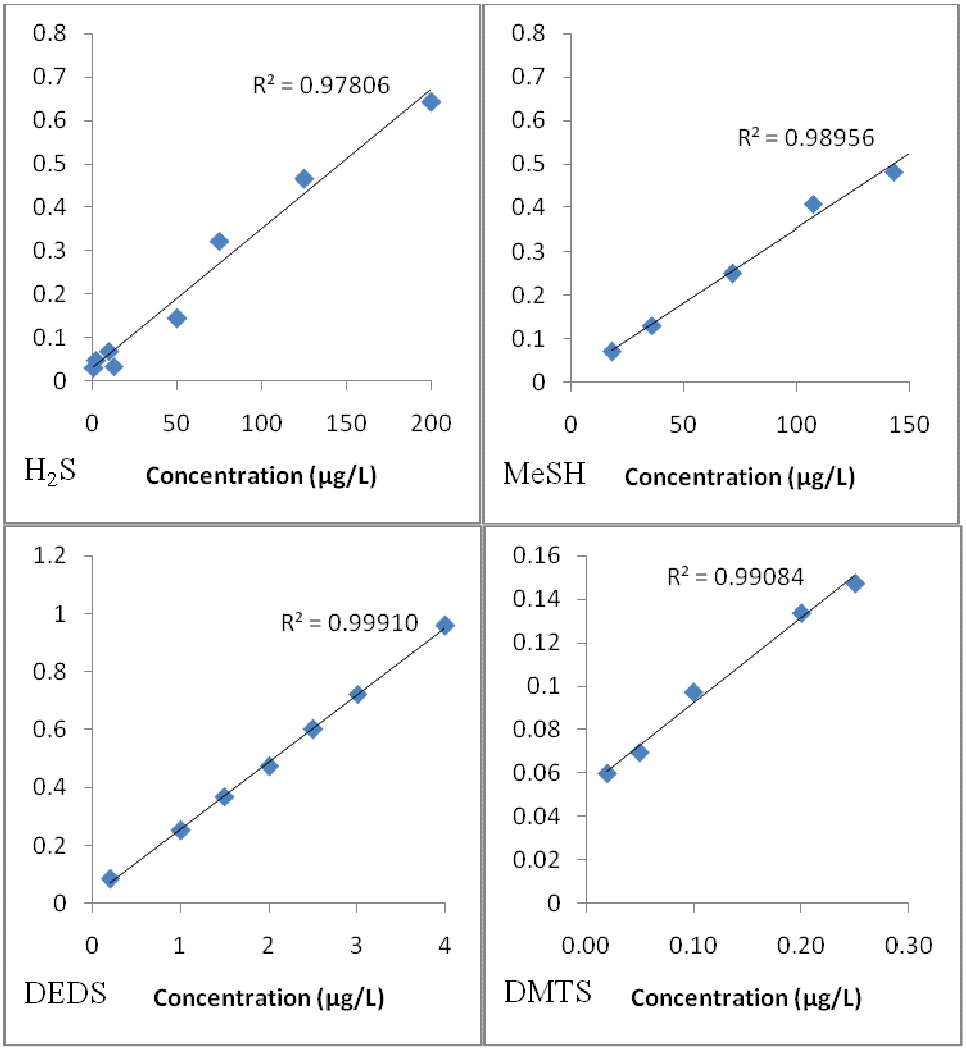


Figure 4.2 (continued): Calibrations curves for sulfur standards; y-axis = ratio of S:IS response (sqrt(peak area)); DMS (IS=EMS); DMDS, MeSOAc, EtSOAc (IS=DES); H_2S, MeSH, DEDS, and DMTS (IS=DIDS)

Table 4.1: Quantification of sulfur volatiles in 21 California wines (µg/L)

Varietal	Year	H_2S	MeSH	DMS	MeSOAc
Chardonnay	2009	2.35 ± 0.290	5.64 ± 0.578	25.12 ± 0.603	6.03 ± 0.091
Chardonnay	blend	19.35 ± 4.513	7.07 ± 0.364	53.02 ± 0.123	nd
Chardonnay	blend	22.25 ± 5.527	6.85 ± 0.508	30.77 ± 0.575	6.67 ± 1.665
Moscato	2010	1.15 ± 0.232	nd	4.08 ± 0.041	24.02 ± 14.116
Pinot Gris	2010	8.92 ± 0.113	0.66 ± 0.081	25.44 ± 0.029	trace
Riesling	2009	1.11 ± 0.148	2.56 ± 0.054	11.48 ± 0.624	nd
Riesling	2009	5.47 ± 1.136	trace	14.53 ± 0.557	nd
Sauv. Blanc	2009	trace	trace	13.59 ± 1.337	15.19 ± 13.451
White (blend)	blend	25.39 ± 3.823	3.31 ± 0.461	17.84 ± 0.997	nd
Cab. Sauv.	2009	trace	1.25 ± 0.097	59.46 ± 0.100	21.38 ± 0.153
Cab. Sauv.	2008	trace	3.21 ± 0.286	84.51 ± 5.822	7.75 ± 0.175
Cab. Sauv.	2007	1.13 ± 0.205	1.95 ± 0.140	55.12 ± 0.011	2.07 ± 0.042
Cab. Sauv.	blend	trace	0.37 ± 0.030	42.04 ± 0.591	19.46 ± 0.048
Malbec	2009	trace	3.70 ± 0.441	45.22 ± 0.938	18.10 ± 0.161
Merlot	blend	trace	1.15 ± 0.039	42.24 ± 1.562	34.00 ± 0.233
Merlot	2009	trace	trace	34.99 ± 0.267	17.90 ± 0.315
Merlot	2008	trace	1.01 ± 0.054	51.26 ± 4.155	11.13 ± 4.129
Pinot Noir	2009	trace	2.87 ± 0.510	19.44 ± 0.562	18.74 ± 0.467
Pinot Noir	blend	9.60 ± 0.761	1.19 ± 0.268	20.28 ± 0.258	22.56 ± 0.144
Zinfandel	2009	trace	2.13 ± 0.210	64.04 ± 0.115	29.28 ± 0.056
Red (blend)	blend	trace	1.52 ± 0.183	33.49 ± 1.897	42.95 ± 0.301

Table 4.1 (continued): Quantification of sulfur volatiles in 21 California wines (µg/L)

Varietal	Year	DMDS	EtSOAc	DEDS	DMTS
Chardonnay	2009	0.16 ± 0.008	nd	trace	0.18 ± 0.020
Chardonnay	blend	0.06 ± 0.004	nd	trace	0.18 ± 0.007
Chardonnay	blend	0.64 ± 0.080	trace	0.01 ± 0.001	0.32 ± 0.041
Moscato	2010	0.02 ± 0.004	2.88 ± 1.966	0.02 ± 0.004	0.03 ± 0.002
Pinot Gris	2010	trace	nd	trace	0.05 ± 0.004
Riesling	2009	0.01 ± 0.001	nd	trace	0.14 ± 0.032
Riesling	2009	trace	nd	trace	0.03 ± 0.002
Sauv. Blanc	2009	1.06 ± 0.025	5.00 ± 0.430	0.08 ± 0.003	0.04 ± 0.000
White (blend)	blend	trace	nd	trace	0.04 ± 0.004
Cab. Sauv.	2009	0.16 ± 0.032	0.71 ± 0.024	trace	0.18 ± 0.007
Cab. Sauv.	2008	0.27 ± 0.028	nd	trace	0.02 ± 0.002
Cab. Sauv.	2007	0.03 ± 0.001	nd	trace	0.02 ± 0.002
Cab. Sauv.	blend	trace	1.62 ± 0.130	trace	0.06 ± 0.004
Malbec	2009	0.48 ± 0.042	0.72 ± 0.017	trace	0.29 ± 0.014
Merlot	blend	trace	2.98 ± 0.021	trace	0.08 ± 0.013
Merlot	2009	trace	0.93 ± 0.085	trace	0.08 ± 0.002
Merlot	2008	0.54 ± 0.145	0.22 ± 0.308	trace	0.09 ± 0.003
Pinot Noir	2009	0.08 ± 0.019	0.39 ± 0.013	trace	0.15 ± 0.043
Pinot Noir	blend	trace	1.52 ± 0.075	trace	0.10 ± 0.050
Zinfandel	2009	0.04 ± 0.000	2.43 ± 0.103	trace	0.23 ± 0.011
Red (blend)	blend	trace	5.68 ± 0.058	trace	0.13 ± 0.022

GENERAL CONCLUSIONS

The analysis of volatile sulfur compounds has posed a challenge because of the volatility and low concentrations often found in food and beverage samples. Various methods have been used to compensate for these challenges, employing techniques and devices to extract and stabilize sulfur compounds from desired sources. SPME has become the standard method used for extracting and concentrating sulfur volatiles, using a solventless sampling method to remove only volatile analytes. Gas chromatography coupled with some type of detection, usually PFPD or chemiluminescence, is used to separate volatiles for analysis.

The wine matrix poses another issue for analyses, as various components within the sample can affect the sensitivity of the SPME technique. Ethanol can greatly decrease sensitivity of sulfur compounds through SPME extraction, as can other volatile components like alcohols, esters, and acids, even at wine concentrations. Limited space for adsorption on the fiber causes competition between compounds, which can lead to displacement of smaller, more volatile compounds, with heavier, less volatile compounds. Ethanol, being present in substantial concentrations in wine, can act as a co-solvent for volatiles, diminishing their ability to pass through the air-liquid interface and into the headspace. However, the non-volatile components of wine (tannins, pigments, proteins, organic acids, etc.) seem to have little effect on SPME extraction.

In order to accurately analyze volatile sulfur compounds, despite matrix parameters, a suitable internal standard must be found that will be affected in

the same manner as the analyte of interest. Three fibers and five internal standards were used to analyze volatile sulfur compounds in synthetic wine matrices in order to determine the best method to compensate for said effects. The carboxen-PDMS fiber showed the greatest sensitivity compared to other fibers. The effects of ethanol on sulfur analysis were very substantial; a great decrease in sensitivity was seen between 0.0 and 0.5%. Ratios of analyte:internal standard were most level between 2-4% ethanol, suggesting a dilution of 2mL wine to 8mL salt water satisfactory for analysis. Both H_2S and MeSH, traditionally analyzed with EMS as internal standard, showed poor resemblance to EMS with increasing ethanol content. DIDS was found to be a more suitable internal standard, despite their dissimilarities, based on their response to the matrix and their behavior on the fiber.

A similar effect was seen with volatile, non-ethanol matrix (acids, esters, alcohols), though the ratios were relatively consistent. However, DMDS, MeSOAc, and EtSOAc, while accurately measured against EIS and DES throughout ethanol changes, were only accurately measured against DES throughout volatile matrix changes. The increase of a devolatilized-wine matrix did not have a significant effect on the sensitivity of sulfur compounds or their ratios with internal standards. Thus, EMS was chosen as internal standard for DMS, DES was chosen as internal standard for DMDS, MeSOAc, and EtSOAc, and DIDS was chosen as internal standard for H_2S and MeSH. Using this method, sulfur compounds were calibrated with good linearity (R^2=0.97-0.99). Quantification of 21 California wines was possible, despite various ethanol contents and volatile profiles.

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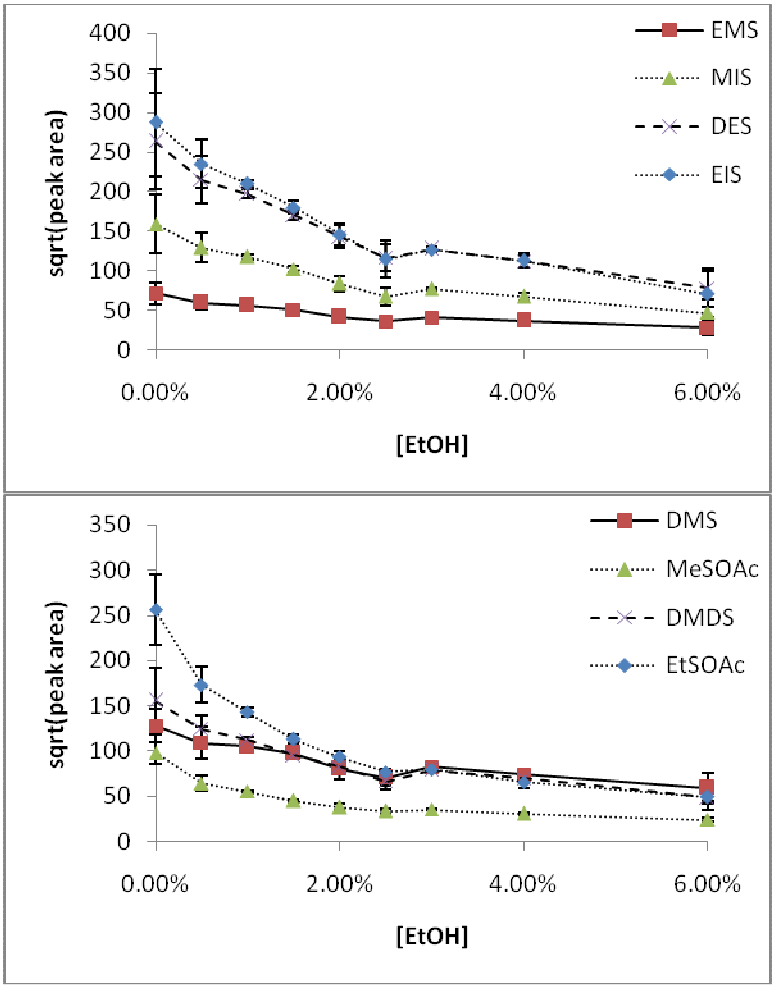
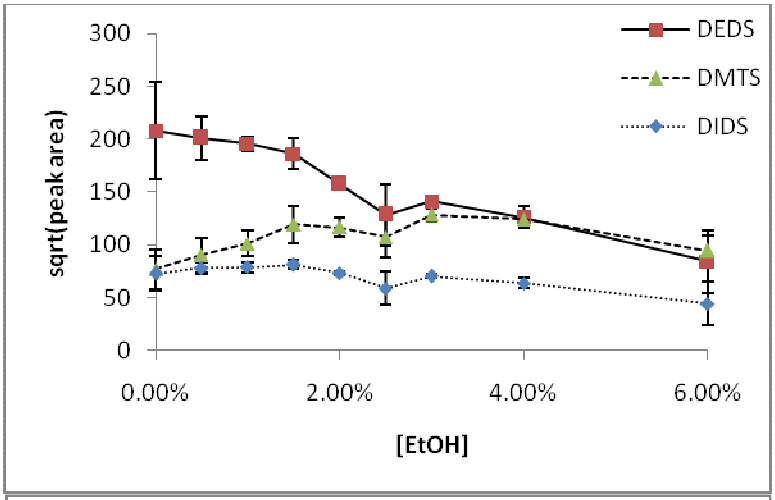
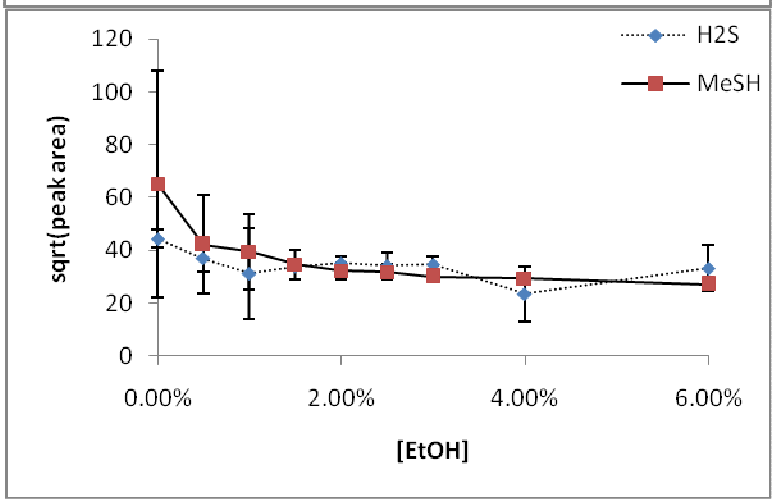
APPENDIX: EFFECTS OF ETHANOL CONTENT ON DIRECT AND RATIO ANALYSES ON CARBOXEN-PDMS AND DVB-PDMS FIBERS

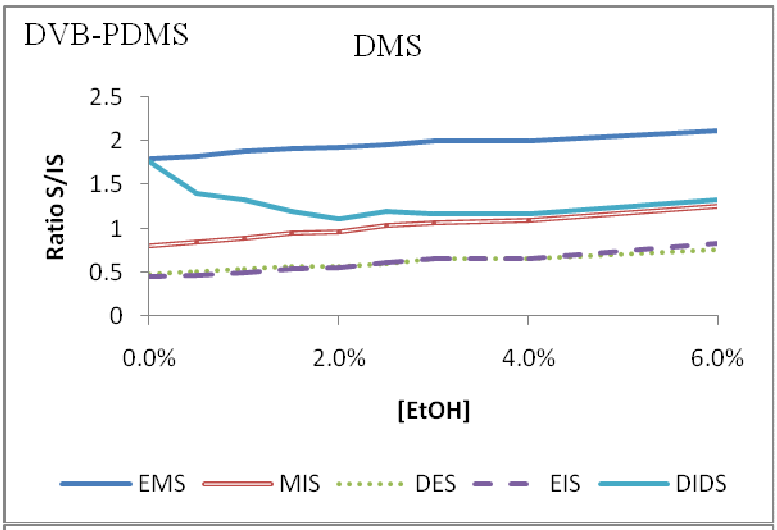
Direct Analysis on DVB-PDMS Fiber

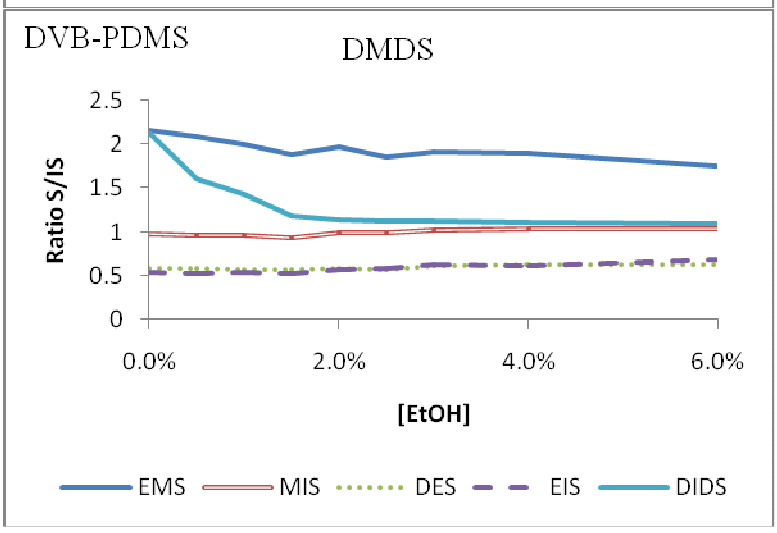


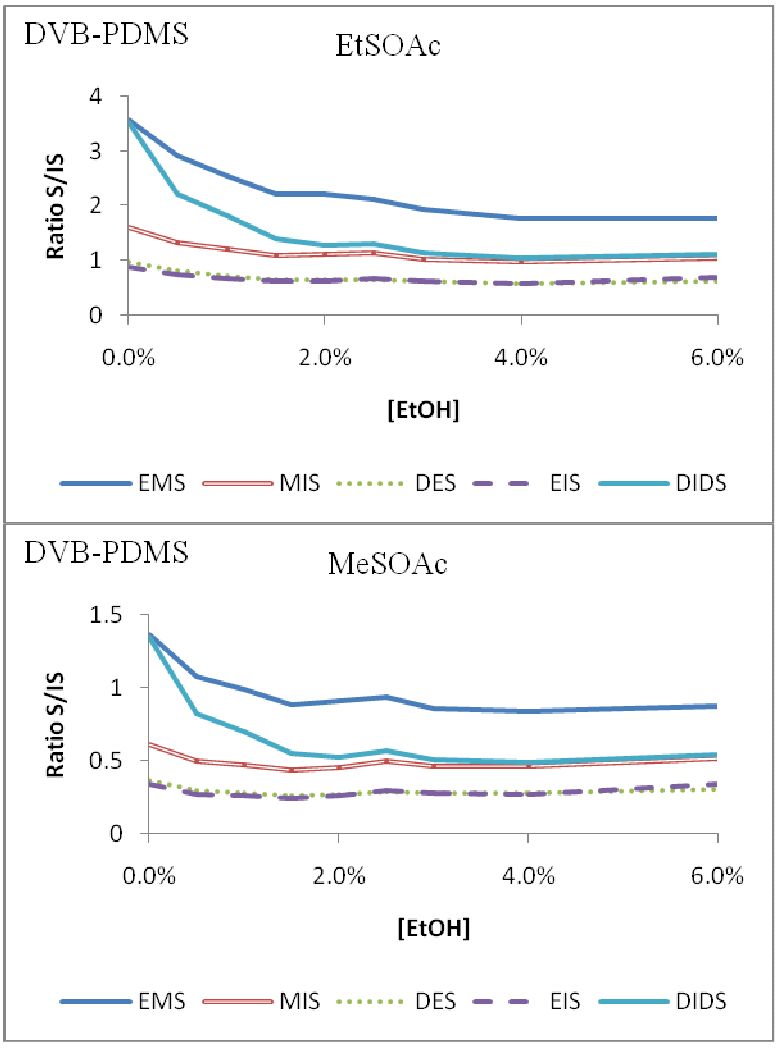

300
250
200
150
100
50
0
sqrt(peak area)
0.00%
2.00%
4.00%
6.00%
[EtOH]
DEDS
DMTS
DIDS

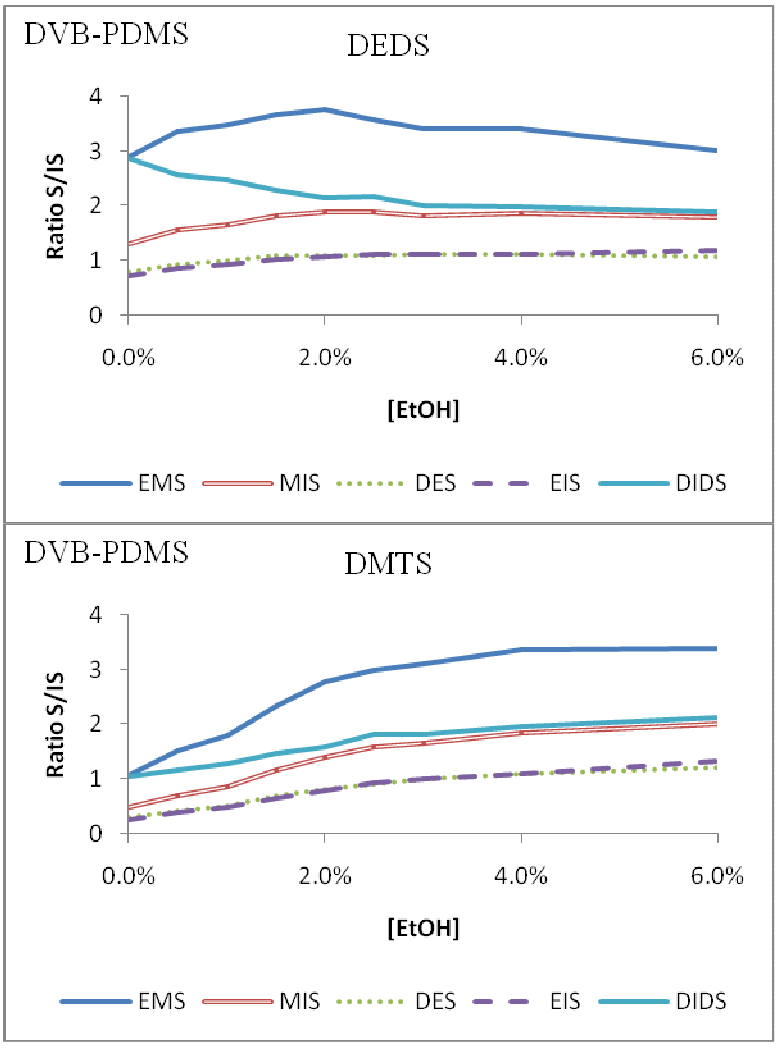

120
100
80
60
40
20
0
sqrt(peak area)
0.00%
2.00%
4.00%
6.00%
[EtOH]
H2S
MeSH

Ratio Analysis on DVB-PDMS Fiber

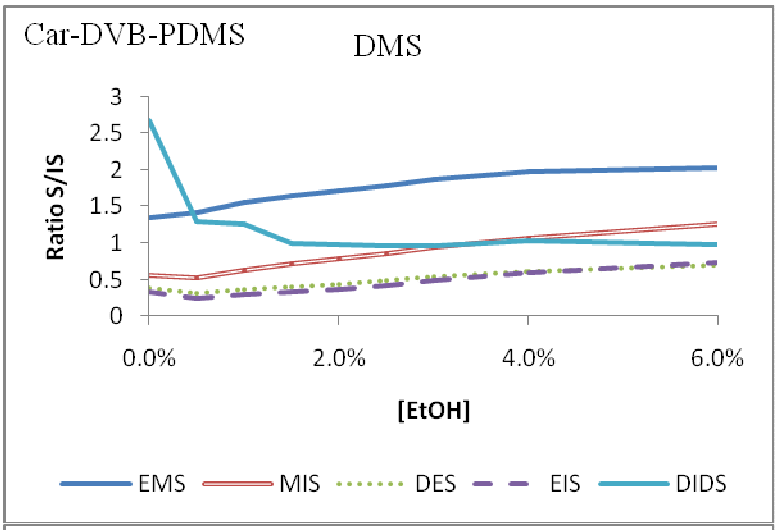


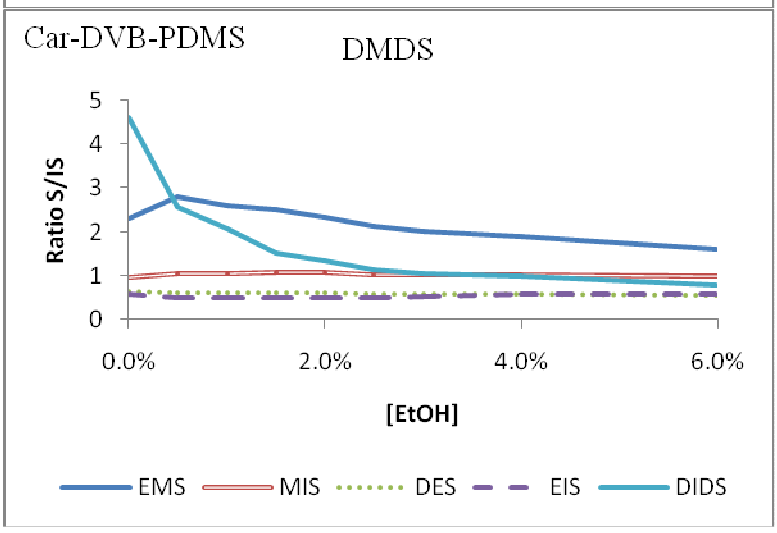


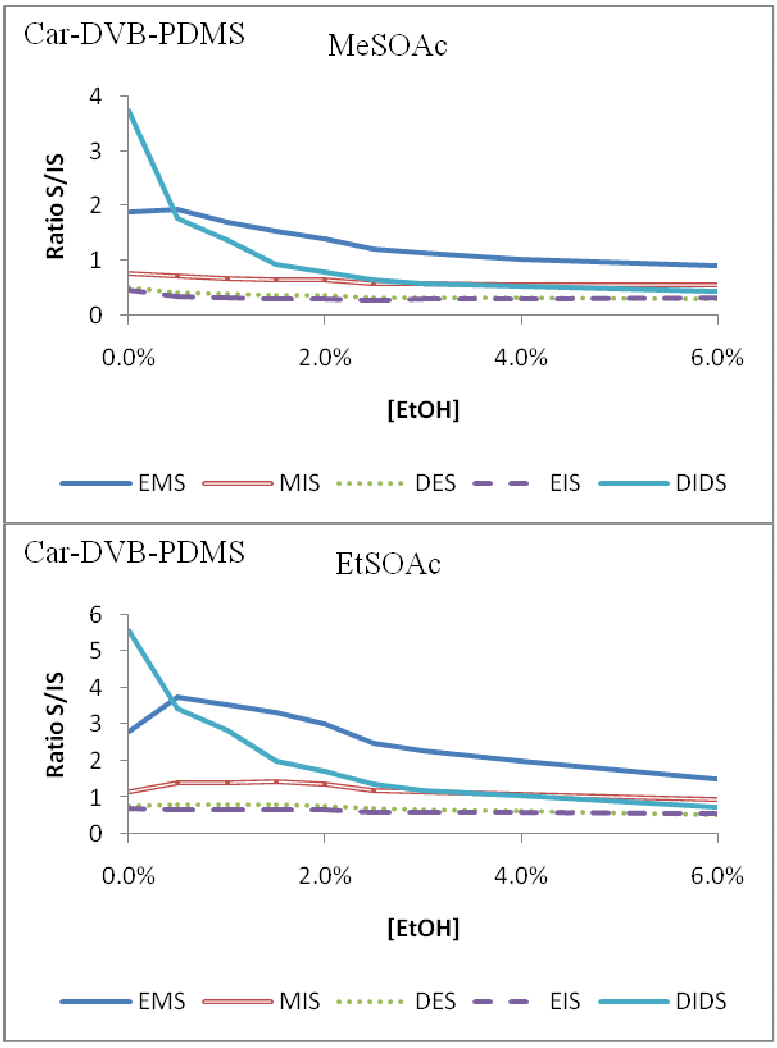

DVB-PDMS
EtSOAc
Ratio S/IS
4
3
2
1
0
0.0%
2.0%
4.0%
6.0%
[EtOH]
EMS
MIS
DES
EIS
DIDS
DVB-PDMS
MeSOAc
Ratio S/IS
1.5
1
0.5
0
0.0%
2.0%
4.0%
6.0%
[EtOH]
EMS
MIS
DES
EIS
DIDS

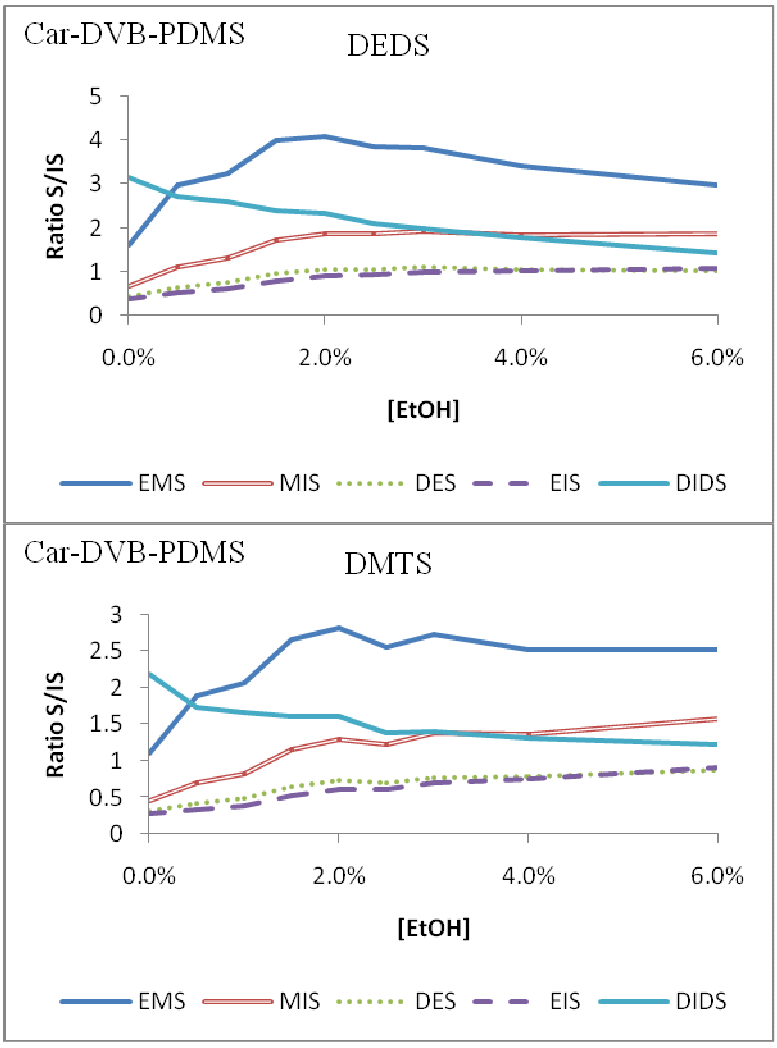

DVB-PDMS
DEDS
Ratio S/IS
4
3
2
1
0
0.0%
2.0%
4.0%
6.0%
[EtOH]
EMS
MIS
DES
EIS
DIDS
DVB-PDMS
DMTS
Ratio S/IS
4
3
2
1
0
0.0%
2.0%
4.0%
6.0%
[EtOH]
EMS
MIS
DES
EIS
DIDS

Ratio Analysis on Car-DVB-PDMS Fiber






Car-DVB-PDMS
MeSOAc
Ratio S/IS
0
1
2
3
4
0.0%
2.0%
4.0%
6.0%
[EtOH]
EMS
MIS
DES
EIS
DIDS
Car-DVB-PDMS
EtSOAc
Ratio S/IS
0
1
2
3
4
5
6
0.0%
2.0%
4.0%
6.0%
[EtOH]
EMS
MIS
DES
EIS
DIDS


Car-DVB-PDMS
DEDS
Ratio S/IS
5
4
3
2
1
0
0.0%
2.0%
4.0%
6.0%
[EtOH]
EMS
MIS
DES
EIS
DIDS
Car-DVB-PDMS
DMTS
Ratio S/IS
3
2.5
2
1.5
1
0.5
0
0.0%
2.0%
4.0%
6.0%
[EtOH]
EMS
MIS
DES
EIS
DIDS