ANALYSIS OF SULFUR COMPOUNDS USING A WATER STATIONARY PHASE IN GAS CHROMATOGRAPHY WITH FLAME PHOTOMETRIC DETECTION

by

Kaylan H. McKelvie and Kevin B. Thurbide*
Department of Chemistry,
University of Calgary,
Calgary, Alberta, T2N 1N4
Canada

Submitted for publication as an Original Research Paper in:
Analytical Methods

*Corresponding Author
Phone: (403) 220-5370
Fax: (403) 289-9488
E-mail: thurbide@ucalgary.ca

ABSTRACT

The properties of using a water stationary phase for analyzing organic sulfur compounds in capillary gas chromatography (GC) with a flame photometric detector (FPD) are presented. The water phase was found to not hinder FPD performance, which provided a detection limit near 30 pgS/s and a selectivity of 3 x 10^4 for sulfur over carbon that agrees well with most commercial devices. Several different organosulfur compounds were examined and found to be retained to varying degrees on the phase. In many cases, analyte water solubility and polarity appeared to correlate well with retention, whereas analyte boiling point did not. By comparison, non-polar hydrocarbons were generally unretained in the system. This prevented their co-elution with sulfur analytes and the response quenching that is often observed in conventional GC-FPD. Of note, when a gasoline sample was analyzed on a standard DB-1 column, the response of the sulfur analytes present was found to be quenched by about 50% due to the overlapping hydrocarbon species also present. However, the same sample analyzed on the water stationary phase displayed no response quenching. Additionally, it was found that sulfur compounds present in different aqueous matrices such as wine, milk, and urine could also be readily and directly analyzed without interference, since many of the large hydrophilic matrix components present are often fully retained on the phase. Results indicate that this method can provide a useful alternative for the analysis of organosulfur compounds in complex matrices.

Keywords: gas chromatography, sulfur, flame photometric detection, stationary phase, water

1. INTRODUCTION

The analysis of volatile organic compounds containing sulfur is important in many areas such as the detection of chemical warfare agents[1,2] and pesticides,[3–5] petroleum refining,[6–9] and food/beverage quality control.[10–12] A common approach used for this purpose is gas chromatography (GC) employing a sulfur-selective detector such as the sulfur chemiluminescence detector[8,13,14] or the atomic emission detector.[15–17] While these devices provide good analytical performance, the relative cost and maintenance associated with them is also often a concern.[18,19] One of the most widely used sensors in this regard is the flame photometric detector (FPD)[20–30] due in part to its high sensitivity and selectivity for sulfur[31,32] along with its rugged design and simple operation.[33] Additionally, the FPD is a relatively inexpensive detector that can also respond selectively to other heteroatoms, such as phosphorus, tin, and several metals.[34]

Despite these benefits, there exist some well-known major disadvantages of the FPD. One is its non-linear response to sulfur, which can complicate analyte quantification.[34] Perhaps its greatest problem, though, is the signal quenching that occurs when analytes co-elute with hydrocarbons, which decreases the observed response and can compromise analytical results.[35] This is most common in complex matrices that contain thousands of different compounds, such as petroleum samples, where the determination of organosulfur analytes can be difficult to achieve using the FPD.[36] One approach to address this issue has been improved FPD designs that can reduce quenching, such as the dual-FPD[37,38] and more recently the multiple-FPD.[39–42] However, the dual-FPD is not always effective in this regard and the multiple-FPD is not yet commercially available.

Alternatively, another means of overcoming this barrier has long been the pursuit of better separation between hydrocarbons and sulfur compounds to prevent such co-elution and response quenching.[43] For instance, many sulfur compounds are often separated using conventional non-polar (e.g. dimethylpolysiloxane)[13,15,17,44–48] or polar (e.g. porous layer open tubular)[49] columns, and efforts to optimize their operating dimensions and conditions can increase separation efficiency.[43] However, while this can lead to improvements, the general effectiveness of this approach is still largely hindered by the limited resolution achievable for most complex mixtures on such columns. As a result, separation methods that can yield higher selectivity for sulfur compounds over other hydrocarbons in such matrices could potentially further facilitate this approach and would be beneficial to explore. For example, efforts in multidimensional GC have been focused on improving sulfur speciation in separations.[21]

Recently, we reported a water stationary phase for use in capillary column GC.[50] The phase demonstrates unique properties such as retention being primarily based on analyte water solubility and little dependant on volatility. Accordingly, non-polar hydrocarbons display almost no retention in this method, while functionalized compounds are relatively well-retained. For example, several oxygenates were selectively analyzed amongst the hydrocarbons in gasolines by this approach, in both the gas and supercritical fluid chromatography modes, each using the flame ionization detector (FID).[50-52] An extensive examination of organosulfur compounds on the water stationary phase has not been reported. However, given its promising attributes, such an investigation with this unique phase would be beneficial to pursue.

This paper explores for the first time the potential of a water stationary phase for analyzing organosulfur compounds, and also examines the system compatibility with selective detection from an FPD. Several analytes and their retention characteristics are examined and

FPD performance when coupled with the water phase is investigated. Various system applications are presented and discussed. The combined selectivity of this approach is found to provide a relatively simple means for direct, quenching-free, and sensitive analyses of such sulfur compounds in complex mixtures.

2. EXPERIMENTAL

2.1 Instrumentation and operation

An HP 5890 Series II GC (Hewlett-Packard, Palo Alto, CA, USA) equipped with an FPD was used in these experiments. The GC system is depicted in Figure 1 and is similar to that described previously with an FID.[50] Briefly, high purity helium carrier gas (Praxair, Calgary, AB, CAN) is bubbled through HPLC-grade water and saturated with vapor (Burdick & Jackson, Muskegon, MI, USA) using a reservoir made from a 1/4" Swagelok cross-union (Calgary Valve and Fitting, Calgary, AB, CAN) connected to a capped stainless steel (SS) tube (4.6 mm i.d. x 5 cm) that resides inside the oven.[50] It is important to emphasize here that this water only serves to saturate the carrier gas and preserve the water phase, which is firmly stationary against the capillary wall and does not move.[51] The carrier gas then passes through a SS pre-heating coil (1/16" o.d. x 250 µm i.d. x 168 cm; Chromatographic Specialities, Brockville, ON, CAN) before entering the injector, which was typically maintained at 220 ºC with a split ratio of 7:1.

The SS capillary column employed (1/16" o.d. x 250 µm i.d. x 30 m; Chromatographic Specialties) was coated with an HPLC-grade water stationary phase (Burdick & Jackson) as described previously[50], which typically yields a phase thickness of about 4 µm. [51] It was then placed inside the oven with the inlet directly connected to the injector. A fused silica restrictor (75 µm i.d. x 50 cm; Biotaq, Gaithersburg, MA, USA) was connected to the column outlet by a

zero dead volume union (Vici-Valco, Houston, TX, USA) and was led directly into the detector where it was situated just below the flame.

The carrier gas velocity was normally maintained at 22 to 26 cm/s. The detector temperature was kept at 320 ºC with flame gases set to 40 mL/min hydrogen (Praxair) and 7 mL/min oxygen (Praxair). Note that although oxygen is used here, air should be useful as an alternative as well. All FPD emission was monitored using a 393 nm optical interference filter (11 nm bandpass; Oriel Instruments, Stratford, USA). It should be mentioned that a useful linear sulfur emission at 750 nm has also been reported [42], and can readily be observed in this system as well. However, since this study was directed toward the vast majority of FPD users that still access the quadratic response at 393 nm and experience the above problems at that conventional wavelength, it was invoked here. For some comparison experiments, a DB-1 column (250 µm i.d. x 30 m; 0.25 µm thickness; Agilent Technologies, Mississauga, ON, CAN) was employed in a conventional unhydrated manner.

2.2 Reagents and supplies

A variety of standard sulfur-containing organic compounds were examined in this study. They include: 2-propanethiol, tetrahydrothiophene (each 97%; Fluka Analytical, Oakville, ON, CAN), tert-butylthiol, 1-propanethiol, 1-butanethiol, dimethyl sulfide, carbon disulfide, diethyl disulfide, dimethyl disulfide, thianaphthene (each 99%; Sigma-Aldrich, Oakville, ON, CAN), 2-butanethiol, diethyl sulfide (each 98%; Sigma-Aldrich), dipropyl sulfide (97%; Sigma-Aldrich), diisopropyl disulfide (96%; Sigma-Aldrich), and 1-hexanethiol (95%; Sigma-Aldrich).

Standard solutions were normally prepared in hexanes (a mix of isomers; EMD, Gibbstown, NJ, USA), except for those in the quenching experiments, which were instead prepared in octane (98%; Sigma-Aldrich) or a commercial automotive fuel (purchased from a

local vendor). Other applications had sulfur solutions prepared directly in wine, milk, or urine samples that were all obtained locally. The urine sample was collected from a healthy volunteer after informed consent was obtained, and all related experiments were conducted in compliance with the relevant laws and institutional protocols established under the auspices of the University of Calgary Biosafety Committee. All other details are outlined in the text.

3. RESULTS AND DISCUSSION

3.1 General operating characteristics

Initial efforts were aimed at establishing the FPD performance characteristics within the assembled system. For example, although no interference was anticipated, it was uncertain if the water-laden carrier gas might adversely impact the detector's background emission and analytical properties. However, upon probing this further, it was indeed found that the FPD yielded favorable and appropriate response behavior. For instance, experiments revealed that with and without the water phase present in the system, the background flame emission intensity remained very low in either case and differed by only 4% over a wide range of system operating temperatures. This was also true of carrier and flame gas flows. Of note, as they were considerably varied during optimizations, the system noise changed very little with and without the water present and only altered on average by a factor of about 1.3. Therefore, no appreciable interference was noted in the detector from the added water vapor present.

Accordingly, good sulfur response was observed with the system. For instance, in terms of performance characteristics, the calibration curve of dimethyl sulfide is shown in Figure 2. As seen, the response obtained increases pseudo-quadratically over about 3 orders of magnitude (roughly 30 pgS/s to 30 ngS/s) and yields a minimum detectable limit near 30 pgS/s. Similar

results were also obtained with other analytes. This response was also quite selective over hydrocarbons at this wavelength, as no signal was observed for dodecane or benzene below amounts of about 150 µg injected on column. This translated into a formal selectivity for sulfur over carbon of about 3×10^4. In all, these values agree quite well with those of conventional GC-FPD methods and most modern commercial manufacturers.[31–33] Therefore, the results indicate that the water stationary phase system can readily interface with an FPD for the analysis of organosulfur compounds.

3.2 Retention characteristics of sulfur analytes

In order to better understand the relative retention characteristics of the system, a number of organosulfur analytes were examined with it. Table 1 shows an example of this with the retention time observed for the various analytes under isothermal conditions of 30 ^{o}C. As seen, the compounds are listed in increasing elution order and they show varying degrees of retention. However, a few interesting trends can be noted from the data.

For example, many analytes show a "normal phase retention pattern" akin to that observed in HPLC, where more polar compounds are greater retained, similar to earlier work with the water stationary phase.[50–52] Of note, this is demonstrated by the elution of sulfides, where the less polar dipropyl sulfide elutes before the increasingly more polar diethyl and dimethyl sulfides. Similarly, the disulfide series elutes in an analogous fashion. Furthermore, in addition to analyte polarity, these elution patterns also trend closely with greater analyte water solubility in many cases. For instance, dimethyl sulfide is nearly 2 orders of magnitude more water soluble than dipropyl sulfide.[53] As well, dimethyl disulfide is near 10-fold more water soluble than diethyl disulfide.[53,54] Note that while this property also implies a potential relationship between analyte retention and Log Kow partitioning, very few values (i.e. only 5)

are available for the analytes studied here. None the less, of those obtained, a good linear relationship between Log Kow and retention was found, with an R^2 correlation of 0.9. Thus, this parameter may be useful to establish in the future as more data becomes available.

In contrast to this, analyte boiling point does not seem to correlate well with retention. For example, also included in Table 1 is the boiling point for each compound. It can be seen that as retention times increase, there is no apparent trend in the corresponding analyte boiling point. For instance, even though dimethyl sulfide possesses the lowest boiling point of 37 ºC, it is more retained than a number of other higher boiling point analytes, including several thiols, sulfides, and even diisopropyl disulfide, which boils at 177 ºC. Additionally, several other similar cases can be seen where this occurs as well. Therefore, in many instances increasing polarity and water solubility appear to be key factors in promoting sulfur analyte retention on the water stationary phase, while boiling point is less relevant. This also agrees well with previous findings for other hydrocarbons on this phase.[50]

Nonetheless, it should be noted that certain thiols did not exhibit this retention behavior. For example, 1-propanethiol was found to elute before 1-butanethiol. Even more odd, 1-hexanethiol eluted between these analytes. However, of the compounds examined, the latter was also the only one to yield a very poor, broad peak shape. This may be due to potential interactions with the stainless steel column wall, as it is well known that some thiols can strongly adhere to such surfaces.[55] In fact, when probing this further, 1-hexanethiol did show some retention on dry stainless steel tubing, whereas other analytes did not. Therefore, it appears possible that such interactions could potentially influence the retention behavior of certain thiols in this system. Still, aside from the adverse separation characteristics noted for 1-hexanethiol, good peak shape and retention behavior was generally noted for the other compounds

investigated here. Figure 3 illustrates this with the separation of some different organosulfur species using the assembled GC-FPD system.

3.3 Reduced FPD quenching

Since addressing FPD quenching was one primary motivation for this study, it was of interest to examine how this may be impacted by the current method. In particular, since most non-polar hydrocarbons are essentially unretained on the water stationary phase, it was anticipated that this might be able to offer beneficial selectivity in cases where peak co-elution can lead to detrimental FPD response quenching. Figure 4a demonstrates this issue for a dimethyl disulfide standard in octane on a conventional DB-1 column. As seen from the octane solvent in the FID trace (left) and the dimethyl disulfide peak in the FPD trace (right), the two co-elute and fully overlap. As a result, the sulfur response obtained is severely quenched and the peak intensity shown is diminished to just 29% of its anticipated value. This is determined by referencing signals against an identical unquenched analyte standard in a non-overlapping hexane solvent on the same column. By comparison, Figure 4b shows the same analysis with the water stationary phase system. As shown, the FID trace (left) displays rapid elution and low retention of the non-polar octane solvent on the phase, still yielding similar hydrocarbon response (within a factor of 1.3) to that obtained in Figure 4a. Conversely, though, the sulfur analyte is retained and well separated from octane. As a result, no hydrocarbon response quenching is observed. Therefore, in complex matrices containing numerous hydrocarbons, this retention behaviour may be potentially useful for alleviating FPD quenching of sulfur analyte signals.

To examine this, a gasoline sample spiked with diethyl sulfide, dimethyl disulfide, and tetrahydrothiophene was also analyzed on a conventional DB-1 column and the water stationary phase. As seen from the FID chromatogram of the DB-1 trial (Figure 5a), the hydrocarbon matrix continually elutes across the 10 minute period displayed. The 3 sulfur test analytes were also found to elute within this same range. As a result, significant analyte signal quenching was observed for this sample in the FPD. Table 2 displays the response erosion that was measured for each analyte, and indicates that about half of the signal was lost due to quenching from overlapping hydrocarbons. In contrast to this, the water stationary phase promotes rapid elution of these same non-polar gasoline components (Figure 5b), and prevents hydrocarbon co-elution and interference with FPD sulfur response as a result. Of note, the data in Table 2 demonstrate that the sulfur signal is essentially fully preserved when the same sample is analyzed on the water stationary phase. Figure 5c further illustrates this with the unquenched FPD sulfur signals obtained from this trial. Therefore, the large bias of the water phase against retaining non-polar hydrocarbons can allow for such components in complex matrices to be completely separated from target analytes and greatly facilitate FPD sulfur analyses.

3.4 Sulfur analysis in other complex matrices

In an analogous fashion, the water stationary phase can also simplify the analysis of other complex matrices that contain a variety of more polar sample constituents. For example, it has been shown previously that highly polar matrix components are often fully retained on the water stationary phase, while more mobile target analytes can be eluted and quantified.[50] Further, there is no subsequent concern for column fouling from the retained species since the water stationary phase can be readily discarded and replenished on demand. Therefore, it was of interest here to also analyze for sulfur in some other challenging matrices using this system.

The first of these was a red wine sample spiked with dimethyl sulfide and dimethyl disulfide. The analysis of these compounds is important since they are often found in wine and can be indicators of bad flavouring if present in high concentrations.[56] However, wine also contains many other components such as sugars, polyphenols, and proteins that increase the turbidity of the product. Therefore, these can often make GC quantification of the sulfur-containing flavour compounds difficult and they frequently necessitate the use of multiple sample preparation steps prior to analysis.[57] As seen in Figure 6, direct injection of the neat wine sample on the water stationary phase results in two prominent peaks for these target analytes on an otherwise smooth background with no other apparent matrix interference. This is also supported by FID traces of the same sample, which are similar in appearance and indicate that many of the other polar and/or high molecular weight components present in the wine remain highly retained on the water phase and do not interfere with the sulfur analysis at hand. Incidentally, while the presence of sulfur dioxide might also be anticipated in such a sample, it was found here to be very highly retained. For example, it did not elute after an hour of observation, even at 100 ^{o}C temperatures using the 30 m column. Therefore, if it were a target analyte in future investigations using this method, the employment of a shorter column could be beneficial.

The second sample investigated was milk, which is subject to similar quality issues when high concentrations of sulfurous compounds are present.[10,11] Additionally, milk can be a very challenging matrix since it is a heterogeneous solution often containing various casein proteins, significant amounts of large triglycerides, and abundant sugars such as lactose, all of which can complicate GC analysis.[10.11] As shown in Figure 7, when a neat injection of milk containing dimethyl sulfide was analyzed on the water stationary phase, a prominent analyte peak is again

observed on an essentially unobstructed background (i.e. no response from large hydrocarbon concentrations breaching the detector selectivity). Therefore, as with the red wine sample, many of the large, polar components in milk appear to be highly retained by the phase, allowing for a relatively simple analysis of the sulfur analyte. This is further confirmed by the FID hydrocarbon analysis of this sample, which shows a very similar trace with the addition of some minor, unretained hydrocarbons that elute early in the separation and do not interfere.

A final investigation focused on the analysis of urine, which is an important area that can facilitate the diagnosis of a number of health issues. For example, decreased levels of urinary dimethyl sulfide have been correlated to instances of breast cancer,[58] while increased dimethyl disulfide concentrations have also been noted as an indicator of skin cancer.[59] Currently, GC analysis of these analytes in such complex matrices can be difficult as urine can contain thousands of metabolites in each sample, including larger components such as steroids, protein hormones, and collagen cross-linker metabolites.[60] Figure 8a demonstrates the chromatogram of a urine sample spiked with dimethyl sulfide and dimethyl disulfide that is injected directly into the water stationary phase system. As seen, these important organosulfur markers are well separated and produce good peak shapes with no apparent background interference from the sample matrix (i.e. no response from large hydrocarbon concentrations breaching the detector selectivity). Again, this is because most of the other components present in the urine are heavily partitioned into the water stationary phase and do not elute from the system. As before, FID hydrocarbon traces of the same sample also further attest to this as little else was detected beyond the target analytes.

Given the strong signals obtained for the above spiked sample, another experiment was performed in efforts to monitor the endogenous formation of such target analytes. Asparagus is

well-known for the pungent odour that it can create in the urine after consuming it, which is due in part to the presence of sulfur compounds such as dimethyl disulfide that evolve during digestion.[61] Therefore, to examine if the system could be able to distinguish such an event at more biologically relevant concentrations, urine was obtained from a healthy individual before and after eating about 500 g of asparagus. As seen in Figure 8b, prior to ingesting the asparagus, no sulfur compounds appear in the urine, which was directly injected into the system. However, after eating it and collecting the urine several hours later for analysis, Figure 8c shows that there is an obvious presence of dimethyl disulfide that arises as a result. This was also evident from the relative odour of each sample. Of particular note, approximately 680 µg of this analyte was determined in the urine sample, which agrees very well with previous reports of near 770 µg of dimethyl disulfide being detected in the same volume of urine by headspace analysis.[61]

Finally, it should also be noted that these separations reproduced quite well as repeat injections of the above samples yielded retention times that differed by about 0.4% RSD (n=3). Therefore, overall the water stationary phase GC-FPD system provides reliable performance that can potentially simplify the analysis of such complex samples by largely preventing matrix interference and reducing the need for sample preparation.

4. CONCLUSION

The analysis of various organosulfur compounds using a water stationary phase GC-FPD system has been described. The FPD demonstrated good compatibility with the phase and yielded figures of merit similar to those of a conventional GC-FPD system. The retention of a number of organosulfur compounds was examined on the column. Many of the analytes showed increasing retention as a function of water solubility and polarity. In all cases, analyte boiling

point was generally a poor predictor of analyte retention. By comparison, most non-polar hydrocarbons are uniquely unretained on the water stationary phase. As a result, the FPD response for sulfur analytes was not subject to conventional signal quenching by co-eluting hydrocarbons, which greatly assists the analysis of complex samples such as petroleum products. Conversely, many large polar molecules are heavily retained on the water stationary phase. Accordingly, this can equally simplify the analysis of complex aqueous samples since they can be directly injected into the system and the sulfur analytes present can be determined with little matrix interference. These results suggest that this GC-FPD water stationary phase system could provide a useful alternative method for analyzing organosulfur compounds in complex matrices.

ACKNOWLEDGEMENT

The authors are grateful to the Natural Sciences and Engineering Research Council of Canada for a Discovery Grant in support of this research.

REFERENCES:

1) K. Ganesan, S. K. Raza and R. Vijayaraghavan, *J. Pharm. Bioallied Sci.*, 2010, **2**, 166–178.

2) Z. Witkiewicz, M. Mazurek and J. Szulc, *J. Chromatogr.*, 1990, **503**, 293–357.

3) S. Berijani, Y. Assadi, M. Anbia, M.-R. Milani Hosseini and E. Aghaee, *J. Chromatogr. A*, 2006, **1123**, 1–9.

4) H. Jing and A. Amirav, *Anal. Chem.*, 1997, **69**, 1426–35.

5) C. Lesueur, P. Knittl, M. Gartner, A. Mentler and M. Fuerhacker, *Food Control*, 2008, **19**, 906–914.

6) M. E. Machado, L. P. Bregles, E. W. de Menezes, E. B. Caramão, E. V. Benvenutti and C. A. Zini, *J. Chromatogr. A*, 2013, **1274**, 165–72.

7) N. E. Moustafa and J. T. Andersson, *Fuel Process. Technol.*, 2011, **92**, 547–555.

8) P. Y. Hsieh and T. J. Bruno, *Energy & Fuels*, 2014, **28**, 1868–1883.

9) C. Yin, H. Li, H. Liu, L. Zhao, Z. Bai, Y. Wang, S. Zhang and C. Liu, *Fuel Process. Technol.*, 2014, **120**, 16–21.

10) Z. Al-Attabi, B. R. D'Arcy and H. C. Deeth, *Crit. Rev. Food Sci. Nutr.*, 2009, **49**, 28–47.

11) K. R. Christensen and G. A. Reineccius, *J. Dairy Sci.*, 1992, **75**, 2098–2104.

12) M. Mestres, O. Busto and J. Guasch, *J. Chromatogr. A*, 2000, **881**, 569–581.

13) I. Al-Zahrani, C. Basheer and T. Htun, *J. Chromatogr. A*, 2014, **1330**, 97–102.

14) L. Mahé, T. Dutriez, M. Courtiade, D. Thiébaut, H. Dulot and F. Bertoncini, *J. Chromatogr. A*, 2011, **1218**, 534–44.

15) D. D. Link and P. Zandhuis, *Fuel*, 2006, **85**, 451–455.

16) D. D. Link, J. P. Baltrus, K. S. Rothenberger and R. C. Striebich, *J. Chromatogr. Sci.*, 2002, **40**, 500–504.

17) Á. Stumpf, K. Tolvaj and M. Juhász, *J. Chromatogr. A*, 1998, **819**, 67–74.

18) R. L. Shearer, *Anal. Chem.*, 1992, **64**, 2192–2196.

19) S. E. Eckert-Tilotta, S. B. Hawthorne and D. J. Miller, *J. Chromatogr. A*, 1992, **591**, 313–323.

20) V. P. Campos, A. S. Oliveira, L. P. S. Cruz, J. Borges and T. M. Tavares, *Microchem. J.*, 2010, **96**, 283–289.

21) E. Engel, J. Ratel, P. Blinet, S.-T. Chin, G. Rose and P. J. Marriott, *J. Chromatogr. A*, 2013, **1311**, 140–148.

22) W. A. Aue and H. Singh, *Spectrochim. Acta Part B*, 2001, *56*, 517–525.

23) X. Lu, C. Fan, J. Shang, J. Deng and H. Yin, *Microchem. J.*, 2012, **104**, 26–32.

24) N. Moreira, P. Guedes de Pinho, C. Santos and I. Vasconcelos, *Food Chem.*, 2011, **126**, 1599–1607.

25) M. Simon, A. P. Hansen and C. T. Young, *J. Dairy Sci.*, 2001, **84**, 774–83.

26) S.-T. Chin, Z.-Y. Wu, P. D. Morrison and P. J. Marriott, *Anal. Methods*, 2010, **2**, 243–253.

27) D. A. Locatelli, J. C. Altamirano, J. M. Luco, R. Norlin and A. B. Camargo, *Food Chem.*, 2014, **157**, 199–204.

28) T. P. Logan, J. S. Graham, J. L. Martin, J. E. Zallnick, E. M. Jakubowski and E. H. Braue, *J. Appl. Toxicol.*, 2000, **20**, 200–204.

29) B. Mitrevski, M. W. Amer, A. L. Chaffee and P. J. Marriott, *Anal. Chim. Acta*, 2013, **803**, 174–180.

30) J. Xiong and B. Hu, *J. Chromatogr. A*, 2008, **1193**, 7–18.

31) J. Sevcik and N. T. P. Thao, *Chromatographia*, 1975, **8**, 559–562.

32) D. F. S. Natusch and T. M. Thorpe, *Anal. Chem.*, 1973, **45**, 1184–1194.

33) S. S. Brody and J. E. Chaney, *J. Gas Chromatogr.*, 1966, **4**, 42–46.

34) M. Dressler, in *Selective Gas Chromatographic Detectors*, Elsevier, 1986, pp. 133–160.

35) D. A. Ferguson and L. A. Luke, *Chromatographia*, 1979, **12**, 197–203.

36) W. Wardencki and B. Zygmunt, *Anal. Chim. Acta*, 1991, **255**, 1–13.

37) P. L. Patterson, R. L. Howe and A. Abu-Shumays, *Anal. Chem.*, 1978, **50**, 339–344.

38) P. L. Patterson, *Anal. Chem.*, 1978, **50**, 345–348.

39) A. G. Clark and K. B. Thurbide, *J. Chromatogr. A*, 2015, **1421**, 154–161.

40) T. C. Hayward and K. B. Thurbide, *Anal. Chem.*, 2009, **81**, 8858–8867.

41) A. G. Clark and K. B. Thurbide, *Can. J. Chem.*, 2014, **92**, 629–634.

42) A. G. Clark and K. B. Thurbide, *J. Chromatogr. A*, 2014, **1326**, 103–109.

43) L. Blomberg, *J. Chromatogr.*, 1976, **125**, 389–397.

44) C. Lopez Garcia, M. Becchi, M. F. Grenier-Loustalot, O. Paisse and R. Szymanski, *Anal. Chem.*, 2002, **74**, 3849–3857.

45) J. Luong, R. Gras, R. A. Shellie and H. J. Cortes, *J. Chromatogr. A*, 2013, **1297**, 231–235.

46) A. Pavlova, P. Ivanova and T. Dimova, *Pet. Coal*, 2012, **54**, 9–13.

47) M. T. Timko, E. Schmois, P. Patwardhan, Y. Kida, C. A. Class, W. H. Green, R. K. Nelson and C. M. Reddy, *Energy & Fuels*, 2014, **28**, 2977–2983.

48) W. Wardencki, *J. Chromatogr. A*, 1998, **793**, 1–19.

49) W. Wardencki, *Encycl. Sep. Sci.*, 2000, 4285–4301.

50) J. A. Gallant and K. B. Thurbide, *J. Chromatogr. A*, 2014, **1359**, 247–54.

51) M. O. Fogwill and K. B. Thurbide, *Anal. Chem.*, 2010, **82**, 10060–7.

52) J. N. Murakami and K. B. Thurbide, *Anal. Chem.*, 2015, **87**, 9429–9435.

53) M. H. Abraham and J. Le, *J. Pharm. Sci.*, 1999, **88**, 868–880.

54) D. Mackay, W. Y. Shiu, K. Ma and S. C. Lee, *Handbook of Physical-Chemical Properties and Environmental Fate for Organic Chemicals*, Taylor & Francis Group, LLC, Boca Raton, FL, Second Edi., 2006.

55) G. Liu, *J. Chromatogr.*, 1988, **441**, 312–315.

56) Y. Fang and M. C. Qian, *J. Chromatogr. A*, 2005, **1080**, 177–185.

57) M. Mestres, C. Sala, M. P. Martí, O. Busto and J. Guasch, *J. Chromatogr. A*, 1999, **835**, 137–144.

58) C. L. Silva, M. Passos and J. S. Câmara, *Talanta*, 2012, **89**, 360–368.

59) J. Kwak, M. Gallagher, M. H. Ozdener, C. J. Wysocki, B. R. Goldsmith, A. Isamah, A. Faranda, S. S. Fakharzadeh, M. Herlyn, A. T. C. Johnson and G. Preti, *J. Chromatogr. B.*, 2013, **931**, 90–96.

60) S. Bouatra, F. Aziat, R. Mandal, A. C. Guo, M. R. Wilson, C. Knox, T. C. Bjorndahl, R. Krishnamurthy, F. Saleem, P. Liu, Z. T. Dame, J. Poelzer, J. Huynh, F. S. Yallou, N. Psychogios, E. Dong, R. Bogumil, C. Roehring and D. S. Wishart, *PLoS One*, 2013, **8**, e73076.

61) R. H. Waring, S. C. Mitchell and G. R. Fenwick, *Xenobiotica*, 1987, **17**, 1363–1371.

442

443 **Table 1: The retention of various organosulfur analytes on the water stationary phase**

Compound	Retention Time (min)	Boiling Point (°C)
carbon disulfide	2.3	46
tert-butylthiol	2.5	64
2-propanethiol	3.0	53
2-butanethiol	3.0	85
1-propanethiol	3.2	68
dipropyl sulfide	3.6	143
diisopropyl disulfide	3.8	177
diethyl sulfide	3.9	92
1-hexanethiol	4.1	153
dimethyl sulfide	4.1	37
diethyl disulfide	4.2	154
dimethyl disulfide	5.2	110
1-butanethiol	5.3	98
tetrahydrothiophene	12.3	121
thianaphthene	25.8	221

*Column temperature is 30 ^{o}C.

Table 2: Preservation[a] of FPD sulfur response in gasoline analyzed on different columns.

Analyte	Conventional DB-1	Water Stationary Phase
Diethyl sulfide	48 ± 9 %	97 ± 3 %
Dimethyl disulfide	57 ± 9 %	105 ± 11 %
Tetrahydrothiophene	45 ± 9 %	109 ± 14 %

a. As a percentage of the original unquenched response of a reference standard in hexane; n = 3.

444

FIGURE CAPTIONS

Figure 1: Schematic diagram of the water stationary phase GC-FPD system.

Figure 2: Calibration curve for dimethyl sulfide response using the water stationary phase GC-FPD system.

Figure 3: Chromatogram showing the separation of various sulfur analytes using the water stationary phase GC-FPD system. The temperature program is 30 ºC for 2 min, then 20 ºC/min to 70 ºC, and then 47 ºC/min to 140 ºC. The elution order is 2-propanethiol, diethyl sulfide, dimethyl disulfide, and tetrahydrothiophene.

Figure 4: The FID (left) and FPD (right) traces of 220 ng of dimethyl disulfide in octane solvent on (A) a conventional DB-1 column and (B) the water stationary phase. Oven conditions are (A) 50 ^{o}C for 2 minutes, then 10 ^{o}C/min to 100 ^{o}C, and (B) 30 ^{o}C.

Figure 5: The FID chromatograms of gasoline spiked with 120 ng of diethyl sulfide, dimethyl disulfide, and tetrahydrothiophene on (A) a conventional DB-1 column and (B) the water stationary phase. The unquenched FPD sulfur signals arising from the latter water phase trial are also shown in (C). Oven conditions are (A) 30 ^{o}C for 1.5 minutes, then 5 ^{o}C/min to 120 ^{o}C, and (B, C) 30 ^{o}C for 4.5 minutes, then 20 ^{o}C/min to 100 ^{o}C.

Figure 6: The FPD chromatogram of dimethyl sulfide (15 ng) and dimethyl disulfide (30 ng) in an undiluted red wine sample directly injected onto the GC water stationary phase. Oven temperature is 30 ^{o}C.

Figure 7: The FPD chromatogram of dimethyl sulfide (30 ng) in an undiluted milk sample directly injected onto the GC water stationary phase. Oven temperature is 30 ^{o}C.

Figure 8: Direct injections of urine in the water stationary phase GC-FPD system. The samples are (A) urine spiked with dimethyl sulfide (15 ng) and dimethyl disulfide (30 ng), (B) unspiked urine obtained before consuming asparagus, and (C) unspiked urine obtained after consuming 500 g of asparagus. Oven temperature is 30 ^{o}C.

474

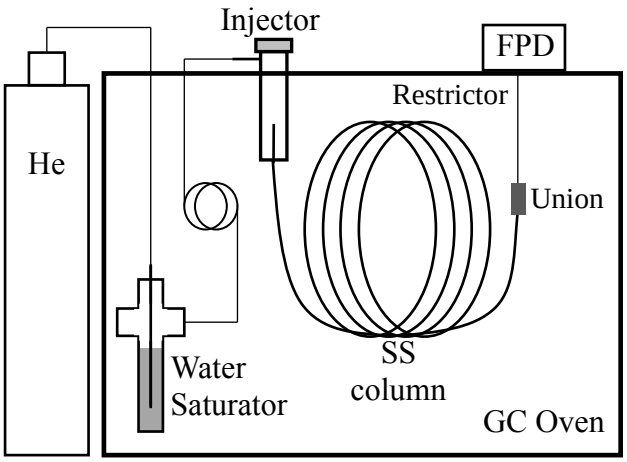
Injector
FPD
Restrictor
He
Union
SS
column
Water
Saturator
GC Oven

Figure 1

475

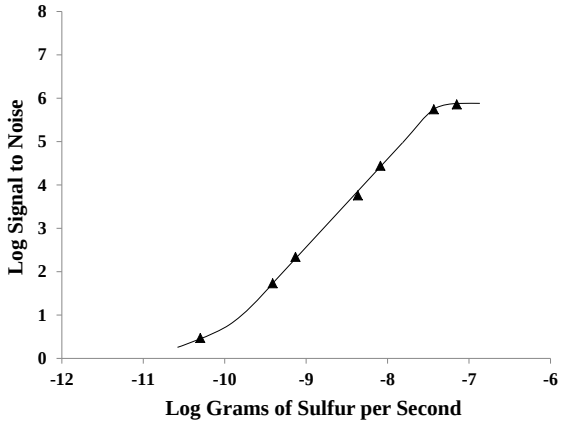
Log Signal to Noise
8
7
6
5
4
3
2
1
0
-12
-11
-10
-9
-8
-7
-6
Log Grams of Sulfur per Second

Figure 2

476

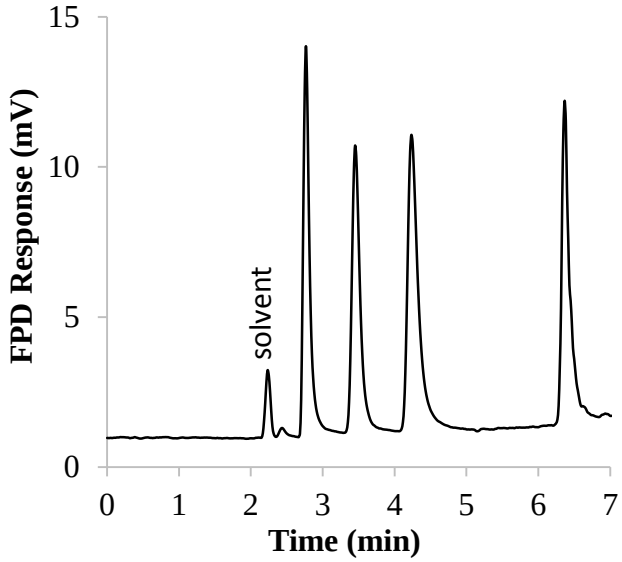

Figure 3

477

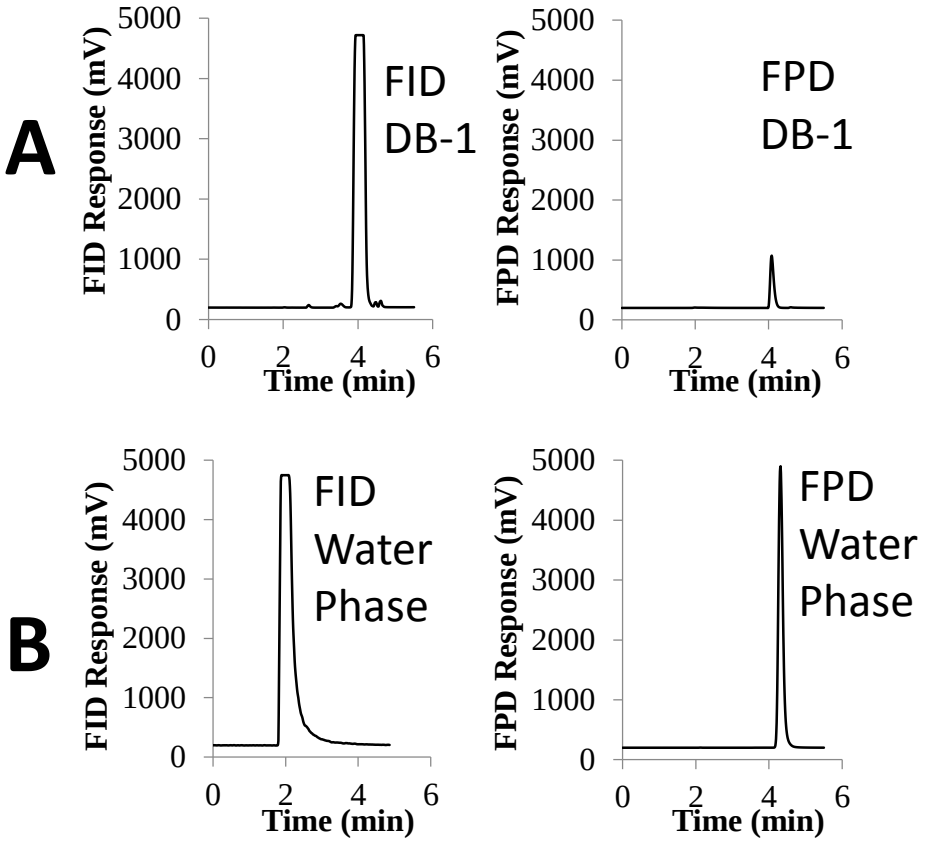
A
FID Response (mV)
5000
4000
3000
2000
1000
0
0
2
4
6
Time (min)
FID
DB-1
FPD Response (mV)
FPD
DB-1
B
FID
Water
Phase
FPD
Water
Phase

Figure 4

478

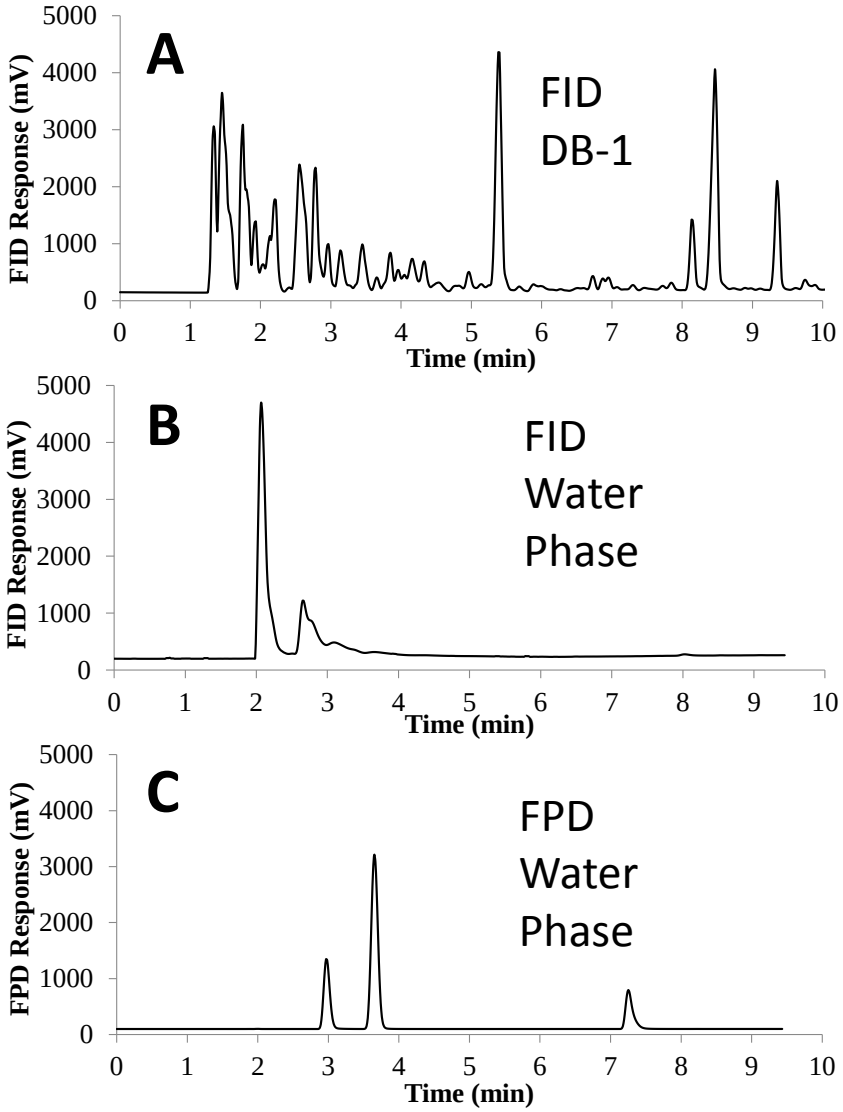
A
FID
DB-1
FID Response (mV)
Time (min)
B
FID
Water
Phase
FID Response (mV)
Time (min)
C
FPD
Water
Phase
FPD Response (mV)
Time (min)

Figure 5

479

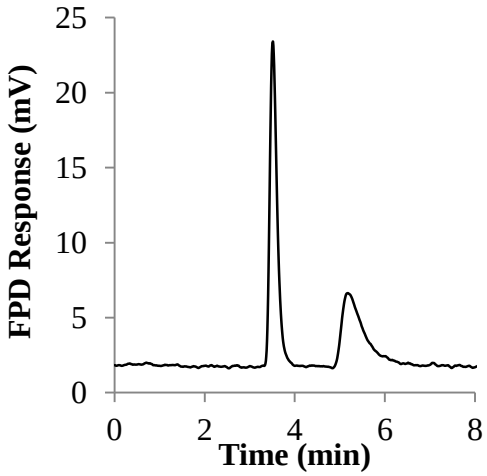

Figure 6

480

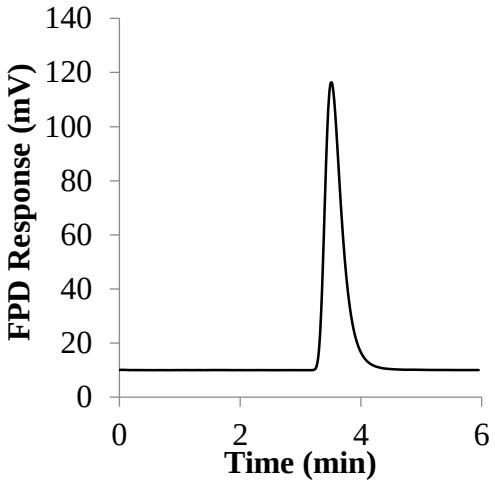

Figure 7

481

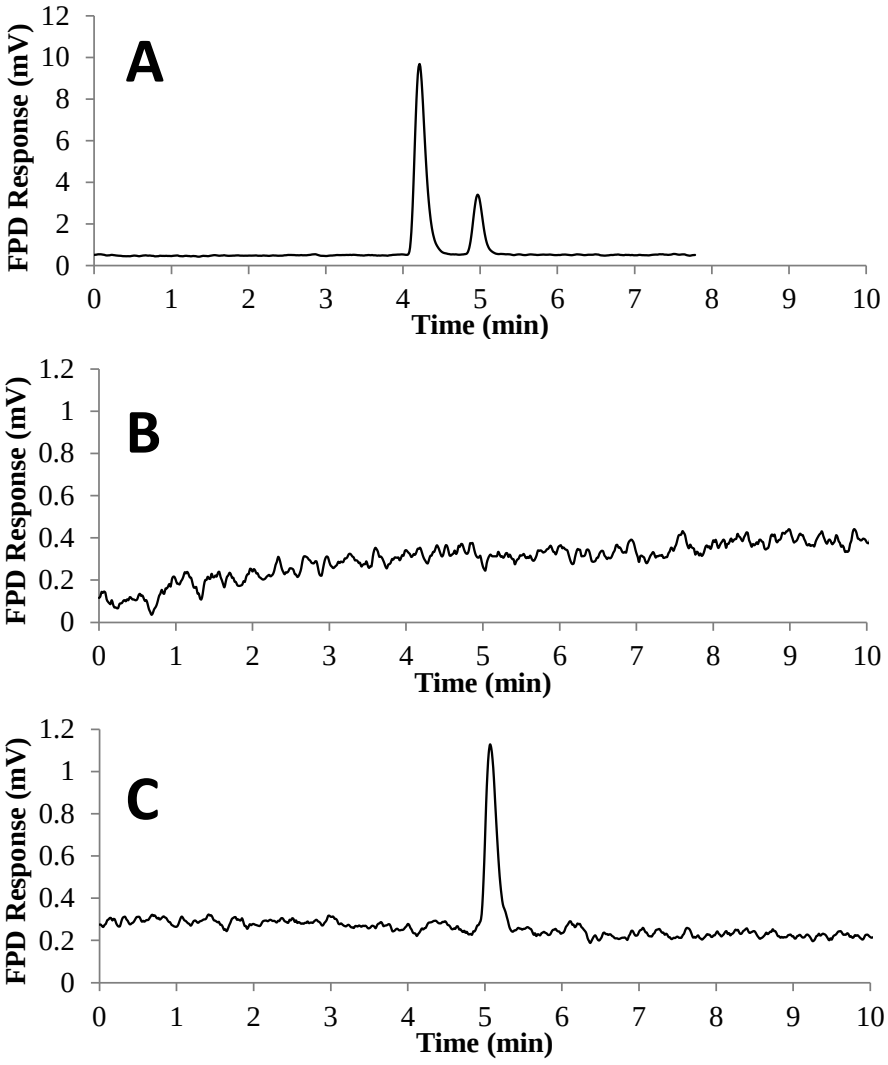
A
FPD Response (mV)
Time (min)
B
FPD Response (mV)
Time (min)
C
FPD Response (mV)
Time (min)

Figure 8