JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Assessment of Thiol Compounds from Garlic by Automated Headspace Derivatized In-Needle-NTD-GC-MS and Derivatized In-Fiber-SPME-GC-MS

Jamie Marcus Warren,[†] Don-Roger Parkinson,*[,§] and Janusz Pawliszyn[†]

[†]Department of Chemistry, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1

[§]Chemistry and Environmental Science, Grenfell Campus, Memorial University of Newfoundland, Corner Brook, Newfoundland, Canada A2H 6P9

ABSTRACT: This study investigates the analysis of thiol compounds using a needle trap device (HS-NTD) and solid-phase microextraction (HS-SPME) derivatized headspace techniques coupled to GC-MS. Thiol compounds and their outgassed products are particularly difficult to monitor in foodstuffs. It was found that with in-needle and in-fiber derivatization, using the derivatization agent *N*-phenylmaleimide, it was possible to enhance the selectivity toward thiol, which allowed the quantitation of butanethiol, ethanethiol, methanethiol, and propanethiol compounds found in fresh garlic. A side-hole NTD was prepared and packed in house and utilized mixed DVB and Carboxen polymer extraction phases made of 60−80 mesh particles. NTD sampling was accomplished in the exhaustive sampling mode, where breakthrough was negligible. This work demonstrates a new application for a side-hole NTD sampling. A commercial mixed polymer phase of polydimethylsiloxane (PDMS) and divinylbenzene polymer (DVB) SPME fiber was used for SPME extractions. Under optimized derivatization, extraction, and analysis conditions for both NTD-GC-MS and SPME-GC-MS techniques, automated sampling methods were developed for quantitation. Both methods demonstrate a successful approach to thiol determination and provide a quantitative linear response between <0.1 and 10 mg L^{-1} (R^2 = 0.9996), with limits of detection (LOD) in the low micrograms per liter range for the investigated thiols. Addition methods using known spiked quantities of thiol analytes in ground garlic facilitated method validation. Carry-over was also negligible for both SPME and NTD under optimized conditions.

KEYWORDS: *thiol compounds, N-phenylmaleimide derivatization, needle trap device (NTD), solid-phase microextraction (SPME), GC-MS*

INTRODUCTION

Of the many important chemical compounds that both meat and vegetable foodstuffs contain, thiol compounds occur naturally but are usually in low concentrations. They are important biological compounds[1] or can be outgassed in growth or through decomposition.[2] Because many thiols are volatile and odor-active, thiol compounds with sapogenins have potential usage in the food-processing sector as natural biomarkers or indicators of food freshness and quality.[3] Importance of thiols for their medicinal properties for human health have been reported.[1,4] For example, garlic is known to contain a variety of thiol compounds that can include monosubstituted thiols (alkylthiols and thiolmethoxide derivatives) and disubstituted thiols (to include rings compounds such as thiophene, as well as the more medically important biological water- and lipid-soluble thiols such as allicin and diallyl disulfide), which have been linked to cholesterol reduction[4] and used for antioxidants.[3] The amounts and kinds of thiol adducts produced can vary in different garlic species and are affected by the conditions under which they are examined, including temperature, which may inhibit enzyme alliinase activity.[2,5] Thiols are difficult analytes to extract and quantitate, and there is a need to find a sampling and extraction technique that will allow thiol substitution differentiation while maintaining careful control of sampling and analysis conditions for quantitation.

The technique of derivatization has been applied to many GC and GC-MS analysis techniques,[6,7] and a review of thiol derivatization[8] is informative. Derivatization offers a number of benefits that include higher temperature stability, improved analyte separation, and signal enhancement for derivatized components (often >100-fold) and hence allow lower detection limits of such compounds to be achieved and increase the working range for calibrative quantitation. For such reasons, derivatization enables quantitative analysis and component selectivity to be carried out on more difficult or complicated matrices, such as in sediment sampling,[9] wastewater,[10] and natural materials, such as foodstuffs,[11] where multiple components in the matrix may mask the target analytes of interest. The derivatization agent *N*-phenylmaleimide has been used to stabilize a number of functional groups on compounds,[12] in particular, for the detection of monothiols (R−S−H) in foodstuffs.[13]

In-fiber derivatization applied to solid-phase microextraction (SPME) offers convenience in the automation of a sampling method, reduces side reactions with the sample matrix, and avoids many problems associated with the addition of the

Received: August 10, 2012
Revised: December 19, 2012
Accepted: December 23, 2012
Published: December 23, 2012

derivatization agent directly to the sample matrix. In-fiber derivatization has been effective using SPME in the headspace mode ((HS)-SPME) for the analysis of ergosterol[14] and lower mass carboxylic acids[15] and for exhaustive extraction of gases.[16]

Another solventless technique that compliments the SPME approach and adds to the analytical arsenal of techniques able to be used in a one-step sampling and extraction method is the emerging technique needle trap device (NTD). The NTD uses a modified syringe that can be doped with a variety of sorbent phases (most frequently composed of polydimethylsiloxane (PDMS), divinylbenzene (DVB), Carboxen, or a mixture of these phases). Previous work has demonstrated the utility of NTD in the analysis of semivolatile compounds in breath[17] and air analysis[18] and for headspace sampling of liquids matrices.[19] This technique has given good linear range with low detection limits, and in some cases, lower detection limits relative to those of SPME have been achieved with this device.[20] It has also been used as a tool to preload derivatization agents for the extraction of airborne carbonyls[18] and dimethylamine.[21] Because NTD is an exhaustive technique, better quantification of target analytes might be achieved by NTD in grab or spot sampling protocols over SPME, an equilibrium technique. However, as both NTD and SPME are solventless techniques, both methods should complement each other and be useful in situations when analyte extraction from complicated matrices is of interest.

Specifically, this paper assesses two analytical derivatized sampling methods, NTD and SPME, to sample five monosubstituted thiol components from fresh garlic (*Allium sativum* L.). Garlic is representative of a foodstuff in which a range of both mono- and disubstituted thiol compounds are known to occur,[4,9] although their detection is often difficult to quantitate.[13] Incorporation of a prederivatization step significantly enhances their respective extractions when analyzed by mass spectrometry. Under automation, full control of optimized conditions can be maintained, allowing the ability of each method to extract and quantitate thiol components from garlic to be compared. The derivatized NTD technique demonstrates a new method for thiol determination in foodstuffs.

MATERIALS AND METHODS

Materials. The thiol compounds (purity) 1-butanethiol (99%), 2-mercaptoethanol (95%), 1-propanethiol (99%), sodium ethanethiolate (80%), sodium thiolmethoxide (95%), and thiophene (90%) used as mass spectrum reference compounds and the derivatization agent *N*-phenylmaleimide (97%) were obtained from Sigma-Aldrich (Milwaukee, WI, USA) and used as-is. Ultrapure water, generated by a Barnstead water purifier (Dubuque, IA, USA), and Analar solvents (Fisher Scientific, Nepean, ON, Canada) were used without further purification. Garlic cloves were obtained from a local grocery and were analyzed fresh as a pealed whole clove and in a freshly julienned clove format. All derivatization, sampling, extraction, and analysis utilized crimped Teflon−Teflon-coated septum sealed cap HS vials. SPME fibers (65 μm PDMS−DVB (Supelco, Bellefonte, PA, USA)) were conditioned prior to use as per the manufacturer's instructions. NTDs were made in house using 22 G × 3.5 in. hypodermic needles (DynaMedical Corp., London, Canada), where DVB-HaySepQ particles 60−80 (Krackeler Scientific, Albany, NY, USA) and Carboxen 1000 particles 60−80 (Supelco, ON, Canada) were used as the adsorbent material phases inside the NTDs.

Preparation of the NTD. An individual NTD (Figure 1) was prepared by inserting a coiled stainless steel wire, inside a 22 G hypodermic needle that had been predrilled to add a small side hole, as outlined by Eom et al.[20] The steel coil was placed at a desired depth (2.5 cm from the tip), by insertion of metal wires from both the top and bottom of the needle. By pushing against the steel coil with the wires, it compressed and formed a plug. Once the steel plug was in place, the tip of the NTD was connected to a flow of ethanol solvent for 2 min. A small amount of 60−80 particle size DVB absorbent phase was made into a slurry with ethanol, and this was injected into the ethanol solvent flow, where it traveled into the needle. The material phase was retained in the syringe by the steel mesh plug. The solvent flow supplied enough force to appropriately pack the sorbent particles to the desired depth. Once packed, the needle was connected to a vacuum aspirator (∼22 mmHg) to remove any excess solvent and to ensure a tight DVB phase packing. The NTD was then placed overnight in an oven (80 °C) to dry. After drying, the NTD hypodermic tip was tapered by connecting the NTD to a drill. As the needle spun in a drill press, a fine grit pressure applicator was applied to the tip for ∼1 min, which tapered the tip to the desired dimensions. It was found that needle tapering prevented excessive septum coring in the GC injector port. Four replicate NTDs were prepared using the above process. Another set of NTDs was prepared to give a total of two phases for examination: a DVB-filled NTD, as well as a sandwich trap using a (50:50) DVB−Carboxen 1000 mixed phase NTD. After the NTDs were prepared, they were conditioned (2 h at 300 °C) to remove any impurities before usage. Each set of four replicate NTDs of the same phase were tested for reproducibility under optimized extraction conditions. This was accomplished by comparing the average GC peak areas of an extracted thiol standard (N = 5 replicates for each needle) for the four needles. In each four NTD phase set, the coefficient of variation was found to be <2%.

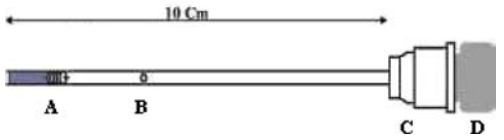

Figure 1. Schematic of the NTD, where A = spring plug, B = side hole, C = needle hub, and D = needle plug.

Instrumentation. A Star 3800 GC-ion trap 2000 MS (Varian, Mississauga, ON, Canada) instrument was used for analysis, where both ionization and mass analysis occur in the same chamber. The GC was equipped with a 1079 split/splitless injector with SPME glass inserts (Varian) and a 5% diphenyl−95% dimethylpolysiloxane HP-5MS fused-silica capillary column (Agilent, Palo Alto, CA, USA) with dimensions 30 m × 0.25 mm i.d. with a 0.25 μm phase. UHP helium was used as the carrier gas at a flow rate of 0.5 mL min^{-1}. Analysis used an experimentally optimized column temperature program: 40 °C, held for 8 min, raised to 150 °C at a rate of 15 °C min^{-1} and to 280 °C at a rate of 40 °C min^{-1}, and held for 20 min, giving a 38.6 min run time. For the MS, the electron multiplier was operating at 2000 V, and all samples were analyzed in electron impact mode (at approximately 70 eV). Mass ranges of m/z 45−350 for thiol reference and derivative compounds and m/z 65−350 for garlic analysis were acquired. The temperatures of the transfer line and ion trap were held at 250 and 180 °C, respectively. Samples were injected into a 250 °C injector port under 10 psi constant pressure. A Combi Pal autosampler (CTC Analytics, Zwingen, Switzerland) with software (version 1.4.0) run on the spectrometer computer controlled all derivatization and extraction conditions for SPME HS sampling.

Thiol Sample Solutions and Derivatization Preparation. Because thiol compounds are smelly and known to be toxic at neat conditions and at concentrations obtained from the manufacturer, a nitrogen glovebag placed inside a fume hood was employed to enable preparation of the standards under a contained N_2 atmosphere. Appropriate dry weight thiols, or volumes of the thiol liquids proportioned using airtight syringes with Teflon coated plungers, were used to prepare the stock thiol reference standards, 1000 mg L^{-1} by dilution with 90% water/10% methanol. When not in use, all stock N_2-purged thiol standard samples were sealed and wrapped in metal foil. Refrigerator storage at −5 °C was found to be adequate, and no alterations occurred over the sampling period. Individual and mixed

dx.doi.org/10.1021/jf303508m | J. Agric. Food Chem. 2013, 61, 492−500

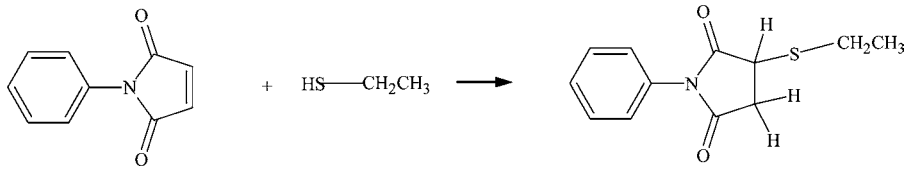

Figure 2. Derivatization scheme showing the reaction of *N*-phenylmaleimide with ethanethiol.

thiol reference standards at concentrations of 10, 5, 2, 1, 0.5, 0.1, and 0.05 mg L^{-1} were prepared, under N_2 in a glovebag, by serial dilution from the stock solutions using (20% NaCl) salt water to give the desired concentrations and thiol combinations. Fresh 10 mL standards in sealed 20 mL SPME HS vials were prepared daily from the stock thiol standards for HS analysis and subsequently analyzed. The individual and mixed thiol standards (10, 1, and 0.1 mg L^{-1}) were used to optimize all conditions and parameters. The derivatization agent sample was prepared fresh before an analysis run by placing about 1 g of the derivatization solid into a N_2-purged amber 20 mL autosampler vial. No derivative decomposition was seen at the end of a sampling day.

SPME Thiol Extractions. Extraction of all thiol solutions (reference standards, spiked and mixed thiol solutions for calibration curve and comparison mass spectral determinations) were performed using an optimized automated derivatized HS-SPME method prior to GC-MS analysis. A variety of temperatures (25, 30, 40, 50, 60, and 70 °C) and agitation rates (500, 600, 700, 750, 800, and 900 rpm) were tested to confirm optimal conditions. For all thiol standard solution samples and garlic samples, the SPME fiber was first derivatized by HS sampling the solid derivatization agent in a 20 mL HS vial for 20 min at 50 °C with a 750 rpm agitation rate. Then headspace extraction of the thiol sample was carried out with the derivatized fiber under the following conditions: HS extraction time, 60 min; extraction temperature, 50 °C; agitation rate, 750 rpm. Before and after each run, the SPME fiber was conditioned for 3 min at 250 °C. No carry-over was noted after such conditioning. As part of the automated method protocol, standards were placed randomly in the run sequence and a fiber blank (to allow monitoring of the fiber integrity) was run as every eighth sample in the run sequence.

NT Thiol Extractions. All exhaustive thiol extractions were performed using an optimized derivatization, sampling, and GC-MS method for NT GC-MS analysis. For NTD extractions, the GC injector port was equipped with a narrow-neck deactivated stainless steel liner (SGE Analytical Science, Fisher Scientific, Canada), and the carrier gas was held at a constant flow of 1.0 mL min^{-1}. The derivatization reagent was loaded onto the NTD by extracting a 60 mL sample volume at a rate of 1 mL min^{-1} at 60 °C for 20 min from the headspace of 1 g of solid reagent. After loading of the derivatization reagent, a 20 mL mixture of the thiol standards was extracted at 0.2 mL $min^{-1}$for 60 min at 60 °C. Because the derivatized thiol standard interaction time is the limiting factor for the derivatization reaction,[13] a slow sampling speed was chosen to ensure enough contact time between derivatization reagent and target thiols. Under similar conditions as described for the SPME method, optimized sampling parameters, temperature, rates, and method protocol were controlled using the autosampler.

As a check of the NTD performance to derivatize and extract thiols, extraction time-weighted averaging (TWA) sampling extractions were carried out on the NTD using a 50:50 DVB−Carboxen mixed phase sorbent and a DVB-only bed. For each phase, two scenarios were examined: without and with preloaded derivatization agent onto the sorbent bed. In all cases, the distance between the coating and the needle tip was constant at 0.2 cm during sampling, so that the sorbent phase had a calculated cross-sectional area of 1.3×10^{-3} cm^2. (A guide wire was used to position, adjust, and fix the depth of the spring plug from the opening of a needle.) This depth was the length of sorbent packed on a NTD if the needle is fully packed; otherwise, packing length can be determined by measuring the remaining depth if partially packed.

Thiol reference analytes were sampled through a Teflon septum needle port (Supelco) on a gas chamber[22] that was attached to a thermostated gas generator, which held the thiol component concentrations at 80 μg L^1 for 1-butanethiol, 95 μg L^1 for ethanethiol, 115 μg L^1 for methanethiol, and 85 μg L^1,for 1-propanethiol and maintained the temperature at 26 °C. NTD TWA measurements were taken at ambient room temperature for contact times of 3, 6, 12, 24, 48, 96, and 168 h.

Garlic Sample Solution Preparation. Approximately 2 g of fresh garlic was weighed and transferred into a N_2-purged 20 mL HS sample sealed vial before analysis. Analyses (N = 3) were prepared, and the garlic was analyzed as a peeled whole and as a peeled finely chopped clove. The garlic samples were analyzed every 6 h over an 18 h period to monitor changes in thiol evolution from the garlic. For method validation, the mixed thiol sample standards at concentrations of 10, 1, 0.5, and 0.05 mg L^{-1} were spiked into the finely chopped garlic sample and were analyzed at the 6 h time interval. The analysis was repeated five times and allowed for estimation of the performance of the techniques and calculation of extraction amounts when compared against nonspiked garlic samples.

RESULTS AND DISCUSSION

General and Derivatization Method Development. Although a number of organic reagents exist to derivatize the sulfhydryl group (S−H), many of these are prone to hydrolysis. From previous work[13] and because the goal was to analyze garlic, which has a high consistency of water, *N*-phenylmaleimide was chosen as it was known not to be so sensitive to hydrolysis reactions. The products formed by this derivatization reagent would be monosubstituted thiol compounds only, would provide stable derivatization, and would aid in increasing separation behavior of such thiols. To further minimize hydrolysis possibilities, a prederivatization scheme of the fiber or of the polymer phase (of SPME and NTD sampling, respectively) followed by headspace sampling of the thiol standards made up in a methanol/water matrix and garlic materials were chosen. A derivatization scheme (Figure 2) is representative of the thiolalkane reactions.

A series of thiol reference standards (1-butanethiol, 1-propanethiol, 2-mercaptoethanol, sodium thiolmethoxide, sodium ethanethiolate, and thiophene) were used to evaluate the two techniques. Table 1 gives their molecular weights, retention times, and monitored fragment ions for evaluation and quantification of the nonderivatized and derivatized analytes. Nonderivatized fragment ions were selected from spectra taken from the MS database (NIST98 MS library) supplied by the Varian GC-MS manufacturer and checked against obtained mass spectra. Derivatized thiol structures were previously assigned[13] and were also checked by obtained MS data. Individual thiol peak areas were determined by extracting the monitored peak ions from the full scan by selected ion monitoring (SIM), to retrieve clear peak area measurements. Thiophene was employed as an internal standard used to estimate the ratios of derivatized compounds to the non-derivatized thiol compound standard peak areas to give percentage derivatization. Furthermore, because thiophene

Table 1. Thiol Standard Reference Compounds and Data from Derivatized HS-SPME-GC-MS[a] and from Derivatized Exhaustive HS-NTD-GC-MS[a]

	nonderivatized peak		
compound	mol wt	retention time (min)	monitored fragment ions,[b] m/z (rel abundance, %)
2-mercaptoethanol	78.13	3.0	47 (62), 48 (60), 60 (49), 78 (44)
1-propanethiol	76.16	2.4	47 (88), 61 (12), 76 (100)
1-butanethiol	90.19	2.8	47 (35), 56 (57), 61 (13), 90 (38)
thiophene	84.14	2.3	58 (59), 84 (69)
sodium thiolmethoxide	70.09	2.4	47[c]
sodium ethanethiolate	84.12	2.3[d]	61[c]

	derivatized peak			
compound	mol wt	retention time (min)	% derivatized	monitored fragment ions,[e] m/z
2-mercaptoethanol	252.31	19.2	78,[a] 80[f]	119, 147, 174, 223, 252
1-propanethiol	250.34	20.2	99,[a] 85[f]	119, 147, 174, 250
1-butanethiol	264.37	20.6	89,[a] 94[f]	119, 147, 174, 264
thiophene		nd		nd
sodium thiolmethoxide	221.26	19.5	99,[a] 85[f]	119, 147, 174, 222
sodium ethanethiolate	235.29	19.8	99,[a] 94[f]	119, 147, 174, 236

[a] $N = 3$ (analysis was carried out using SPME with a commercial 65 μm PDMS−DVB fiber). [b] MS fragment ions were determined from known thiol mass spectra NIST98 MS library, Varian Instruments, and checked by experimental data. [c] Estimated, scanned mass range m/z 45−350; separation of underivatized compound in GC was not complete. [d] Estimated. [e] MS fragment ions were determined from known derivatized thiol mass spectra (ref 13) and checked by experimental data; nd, not derivatized by *N*-phenylmaleimide (mol wt, 173.17; retention time, 17.5 min). [f] $N = 3$ (analysis was carried out using the NTD with a 50:50 DVB−Carboxen mixed phase sorbent bed; with a needle phase stability of RSD ~ 3% for over 100 extraction repetitions).

could not be derivatized by the reagent,[13] this compound was included as a check to ensure that not all compounds were being indiscriminately derivatized under the elevated sampling temperature conditions (50 and 60 °C, respectively) used in SPME and NTD sampling methods. Under such conditions the evolution of hydrogen sulfide or the formation of a disubstituted *N*-phenylmaleimide sulfide, which would suggest[8,23] that other sulfur compounds were decomposing or breaking down, was not seen. Perhaps such decompositions were avoided or diminished by pre-nitrogen purging the sample vials before addition of the garlic to the sample, slowing oxidation reactions. For both analytical methods, over ~80% derivatization could be achieved. On average, of three replicate samples, SPME produced better extraction for most target analytes (>5% for some thiols) when the mixed standard solutions were analyzed. The peak enhancement obtained by derivatization, often with height increases of >100 times as compared to nonderivatized peaks, allowed for easy elucidation of the thiol components from other analyte peaks attributed to the matrix. No apparent decomposition of the derivatization reagent was noted after repeated and prolonged (2−3 h) heating time usage in multiple sample analysis runs. The resulting total ion chromatogram of the extracted derivatization agent produced one main peak with minimal extraneous peaks, and hence a relatively clean spectrum was obtained.

Optimization of SPME Extractions. Using mixed-thiol reference standards, a series of different temperatures (25, 30, 40, 50, 60, and 70 °C) with a variety of agitation rates (500, 600, 700, 750, 800, and 900 rpm) were tried to find the optimal volatilization conditions for maximum loading of the derivatization reagent to the SPME fiber. When the solid derivatization agent was heated in a sealed 20 mL HS vial for 20 min, at 50 °C and with 750 rpm agitation rate prior to exposure of the SPME fiber, maximum loading uptakes were achieved. The in-fiber derivatization technique provided reproducible derivatization of the extraction fiber (found <2% coefficient of variation in the main derivatized peak area was achievable for $N = 5$ replicates). This technique also reduced the number of steps needed in the automated method development procedure.[14]

Attempts to use direct sampling of the thiol standard solutions were found to drastically reduce the amount of analyte extracted through predecomposition of the loaded derivatization agent on the fiber by the solvent. Such findings concur with data reported previously[13] and, hence, HS extraction by the derivatized fiber method was chosen to sample thiol compounds. On the basis of the literature,[9,14,15] which suggests that electrolyte saturation would help to increase the ionic strength of the solvent and increase the extraction ability of the fiber, NaCl was added to the matrix solvent. The largest increase of 15% peak enhancement was obtained with the addition of 20% salt to the matrix. Under these conditions, an extraction time of 40 min was found to be adequate for the analysis of mixed thiols. When garlic cloves and spiked garlic analysis were undertaken, slight extraction improvement was achieved when a 60 min extraction time was used under the same conditions. Hence, a 60 min extraction time was used in all subsequent SPME analyses. Longer extraction times did not affect extracted amounts for the thiols. Results are shown in Table 1.

TWA NT Derivatization. Derivatization conditions for the thiols optimized by the SPME method were used as a starting place to obtain optimal NTD conditions. However, to ensure that the amount of sorbent coating in the NTD would not be a contributing factor and limit the amount of thiol analyte mass acquired during the extraction/derivatization process, a series of time weighted average (TWA) experiments were carried out. Two phases for the NTD were investigated: a 50:50 DVB−Carboxen mixed phase sandwich and one that was entirely DVB filled. A pure Carboxen phase was not investigated in this study as it was known that this phase would give broadened peak shapes and extensive tailing, which would have further been exacerbated with the derivatization agent application.

For NTD TWA experiments, the needle trap device was prepared with a previously mentioned fixed gap of 0.02 cm[20,22] between the sorbent end and the needle tip. Individual thiol analyte sample loading times (to cover the time range from 3 to 168 h)[22] were collected from a prepared standard mixture of thiols, which was generated in a gas chamber and analyzed under the otherwise optimized NTD-GC-MS method. The test was performed under conditions with and without prederivatization of the NTD sorbent phase. Optimum loading time assessments were quantitated by examination of relative GC peak areas for the different thiol standards, obtained from the

Table 2. Reproducibility of Loading Derivatization Agent and Target Thiols Using Exhaustive HS-NTD GC-MS, with a 50:50 DVB−Carboxen Mixed Phase Sorbent Bed

	N-phenylmaleimide $N = 25$	methanethiol $N = 5$	ethanethiol $N = 5$	propanethiol $N = 5$	butanethiol $N = 5$
av area counts	970000	230000	360000	260000	280000
%RSD	3.1	6.7	8.3	9.5	8.1

Table 3. Evaluation of TWA-NTD GC-MS Analysis for Loading Times of 12−168 h[a]

compound	linear regression eq (derivatized)	R^2	linear regression eq (underivatized)	R^2	loading rate increase (%)
1-propanethiol	$y = 4390x - 71700$	0.994	$y = 154x - 2500$	0.995	4910
1-butanethiol	$y = 3050x - 25600$	0.995	$y = 82x - 92$	0.992	9410
methanethiol	$y = 6520x + 128000$	0.992	$y = 130x + 2570$	0.993	2750
ethanethiol	$y = 761x + 16020$	0.994	$y = 8x + 176$	0.996	3620

[a] $N = 5$ for all samples. Analysis was carried out using the NTD with a 50:50 DVB−Carboxen mixed phase sorbent bed, with a needle phase stability of RSD ∼3% for over 100 extraction repetitions.

different loading time data. Table 2 gives results obtained from these experiments with the 50:50 DVB−Carboxen mixed phase and demonstrates that good reproducibility in needle loading of the derivatization agent was achieved. The data imply good robustness and technique reproducibility and needle fabrication reproducibility over the numerous replicates. Further testing (also reflected in previous work[20]) verified that the NTDs were stable and reproducible over 100 extraction repetitions with the same needle and gave an RSD ∼3%.

To ensure no components were lost through diffusion to the surroundings during sampling or transfer prior to desorption in the GC port, a zero sink test was performed. A sorbent material is said to act as a zero sink when the acquired analyte components are stable and not affected by manipulations or through the mass loading rate of additional analytes onto the fiber. Zero sink tests were carried out on the NTDs employed, using a thiol solution containing the four thiol reference compounds, under the conditions as specified for TWA analysis.[20,24] Essentially, the NTD was exposed for a specified duration to the mixed-thiol standard. Then the NTD was placed into and exposed to a clean sealed vial (usually N_2 purged) for the same amount of time. The sample was analyzed by GC-MS for determination of its analyte peak area. This result was then compared to a sample that had been exposed to the thiol analyte mixture for the same amount of time but then directly analyzed. The process was repeated for different times (to cover the sampling and loading time range, 12−168 h) on extracted thiol components with and without derivatization. If no differences were found in the amount of analyte sorbed areas between the dual analyses, then the phase was acting as a zero sink. Results from the zero sink test (Table 3) found that the *N*-phenylmaleimide reagent when combined with the 50:50 (DVB−Carboxen) mixed phase behaved as a zero sink for all thiol compounds studied, whereas the DVB phase did not. Increased affinity and capability were achieved with the mixed DVB−Carboxen fiber phase over the tested 12−168 h loading time frame, and the method was able to linearly extract compounds over the tested time frame. These findings compliment other research that has demonstrated that Carboxen, as a sorbent phase, can act as a zero sink for BTEX compounds.[24] Derivatization was found to increase thiol affinity to the sampling phase of the NTD and increase the ability of the NTD to selectively extract the thiol compounds. Under derivatization conditions, the amount of analyte sorbed is dependent upon the product of the volume of the extraction phase, the reaction rate constant, and the distribution constant, as well as the concentration with respect to time.[24] A derivatized phase shifts such affinity equilibria toward extraction, and hence loading rates were found to increase >40-fold when compared to TWA experiments using no derivatization agent. Experimental data in Table 3 suggest that the loaded sorbent allows analytes to diffuse through and react with the reagent simultaneously. To maximize extraction concentrations, a slow sampling speed was chosen to increase contact time between derivatization reagent and target thiols, even though, theoretically, and on the basis of the sampling diameters and surface area used, one could use faster rates.[20] Hence, the derivatized 50:50 DVB−Carboxen combination was used for NTD extractions for all garlic samples to ensure that the lowest detection limits could be achieved.

Optimization of NT Sampling. To accommodate the NTD 10 cm needle length and to ensure proper placement, a larger vial volume (headspace length) was needed to perform the derivatization reaction of the NTD phase. To achieve similar volatility of the derivatization reagent as for SPME, the temperature was also increased. Hence, optimum derivatization conditions required using a sealed 60 mL vial at 60 °C with a 750 rpm agitation rate for 60 min in NT derivatization. No deleterious effects to the derivatization reagent were noted with the 10 °C higher temperatures. To ensure that quantifiable detected peaks could be observed for the prepared thiol standards using the optimized method, a volume of 20 mL was chosen for extraction. To test for needle breakthrough, two NTs were placed in series so that the headspace extraction volume would be taken up through both needles. If the second NT was contaminated with analyte, then the analyte would have been migrating to the second needle, defined as needle breakthrough. The dual needle system was tested with the derivatization agent by extracting combinations of thiol standards. The optimized HS extraction conditions were an extraction volume flow rate of 0.2 mL min^{-1} for a 20 mL total volume at 60 °C. For all analytes tested, no breakthrough was seen at 20 mL extraction volumes. Only when the extraction volume was increased to 30 mL was any appreciable needle breakthrough seen. Hence, a 20 mL volume was chosen for the thiol analysis. To optimize the sampling rate, 0.2, 1, and 10 mL min^{-1} values were tested, using the 1-propanethiol standard. Selection of the optimum rate was accomplished by comparing the amount of standard that was derivatized and extracted, by monitoring the derivatized GC relative peak area for the assigned thiol standard, at each of the different sampling rates. Figure 3 demonstrates that 0.2 mL min^{-1} produced the largest

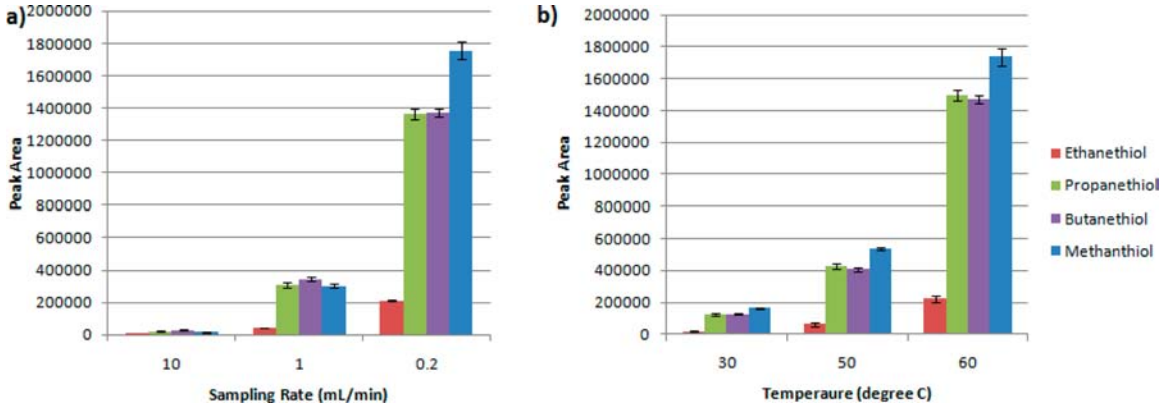

Figure 3. Thiol derivatives as a function of (a) sampling rate and (b) temperature using in-needle derivatization experiment with *N*-phenylmaleimide as the derivatization reagent.

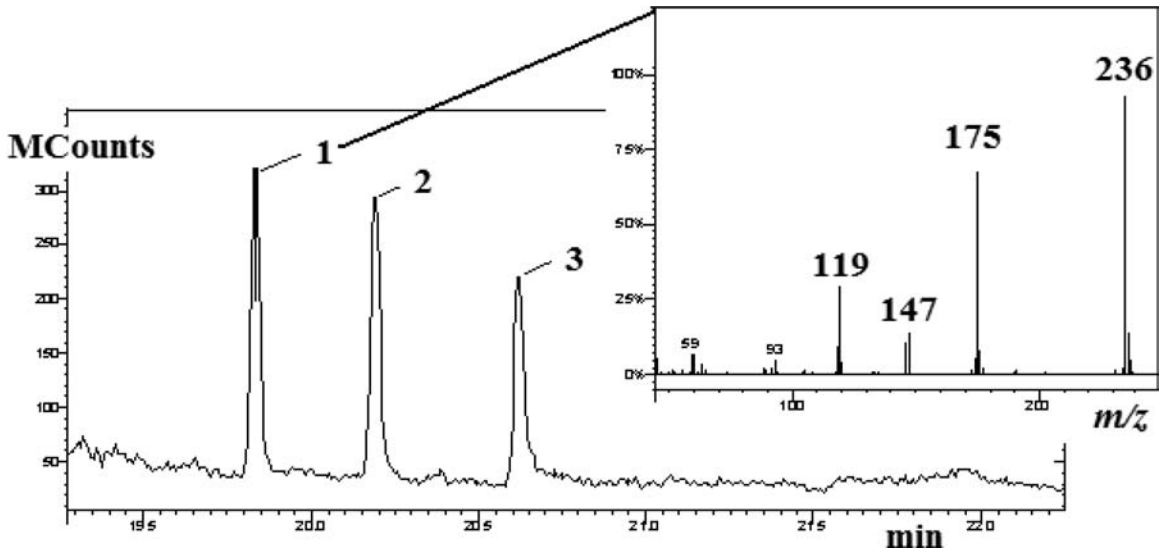

Figure 4. GC chromatogram of three derivatized thiol reference standards using derivatization HS-NTD-GC-MS analysis: (1) sodium ethanolate, derivative 19.8 min (mass spectrum insert); (2) propanethiol derivative, 20.2 min; (3) butanethiol derivative, 20.6 min.

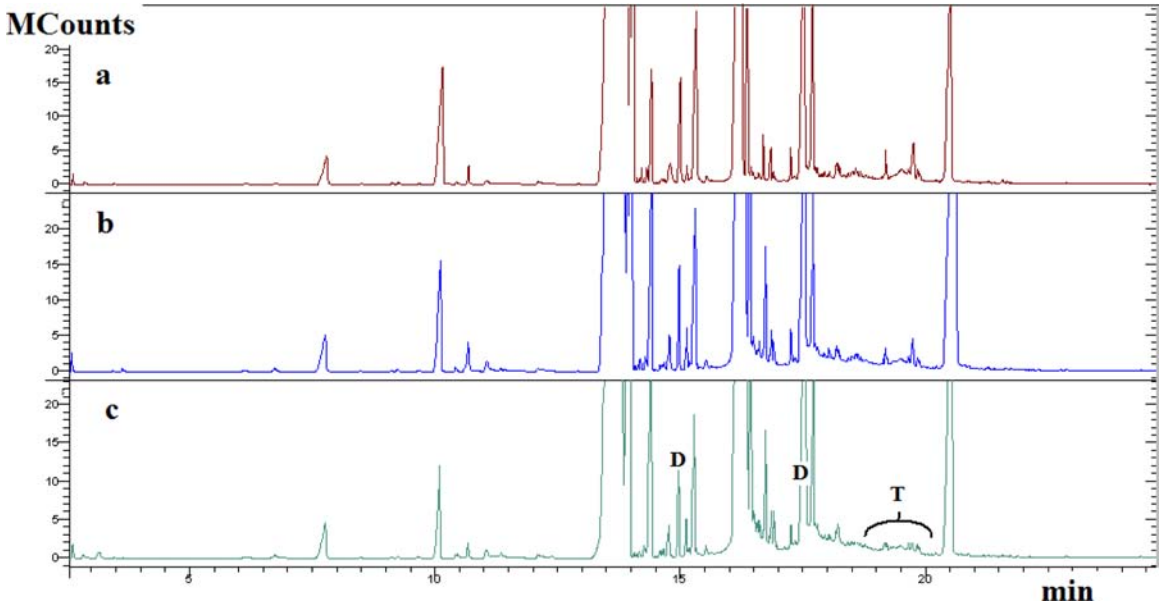

Figure 5. GC chromatogram obtained sampling whole garlic using derivatization HS-SPME-GC-MS after (a) 6 h, (b) 12 h, and (c) 18 h: (T) range of thiol derivative compounds investigated; (D) derivative reagent residue peaks (15 and 17.5 min).

dx.doi.org/10.1021/jf303508m | *J. Agric. Food Chem.* 2013, 61, 492−500

Table 4. Detection Limits and Quantitation Ranges for Derivatized HS-SPME and HS-NTD GC-MS Analyses

SPME				
compound	linear regression eq	LOQR[a] (μg L^{-1})	R^2	detection limit[b] (μg L^{-1})
2-mercaptoethanol	$y = 192000x + 36900$	<0.1−10	0.997	9
1-propanethiol	$y = 244000x + 145000$	<0.1−10	0.999	9
1-butanethiol	$y = 917000x - 99600$	<0.1−10	0.999	10
sodium thiolmethoxide	$y = 522000x - 188000$	<0.1−10	0.997	6
sodium ethanethiolate	$y = 192000x + 36800$	<0.1−10	0.999	9

NTD					
compound	linear regression eq	LOQR[a] (μg L^{-1})	R^2	detection limit[b] (μg L^{-1})	detection limit[c] (mg L^{-1})[a]
1-propanethiol	$y = 169000x + 226000$	<0.1−10	0.998	13	1.80
1-butanethiol	$y = 164000x + 266000$	<0.1−10	0.999	15	0.85
sodium thiolmethoxide	$y = 302000x + 179000$	<0.1−10	0.998	11	2.90
sodium ethanethiolate	$y = 260000x + 308000$	<0.1−10	0.999	15	2.40

[a]LOQR, limit of quantitated range. [b]Detection limit (μg L^{-1}) of derivatized thiols. [c]Detection limit of nonderivatized thiol compounds; $N = 3$ for all samples; SPME fiber, a commercial 65 μm PDMS−DVB fiber; NTD phase, a 50:50 DVB−Carboxen mixed phase sorbent bed.

derivatized thiol component. Hence, sampling rate is an important variable to control, as it denotes that contact time between analyte and sorbent loaded with derivatizing agent is a critical and limiting factor in the derivatization of thiols. For NTD, as with SPME, an extraction time of 60 min was optimal. Figure 4 shows an expanded portion of the TIC obtained by derivatized HS-NTD-GC-MS for three thiol standards used. Table 1 shows data obtained for the extraction of selected thiols found in garlic had high (80%+) derivatization rates using the NTD method. For example, a derivatization/extraction efficiency of 85% was obtained for the 1-propanethiol standard. The percentage efficiency given (averaged over three replicates) was determined by GC peak area comparison of underivatized to derivatized, where their sum was assumed to be 100%.

Analysis of Thiol Standards and Garlic Samples. Under optimized conditions for analysis (see Materials and Methods for particulars), the retention times for the target thiol compounds are given (Tables 1 and 2) and were taken from their corresponding total ion chromatograms. For both SPME and NTD methods, the GC-MS total run time was ∼38 min. All thiol analytes were completely separated by the 22 min stage (Figure 5); however, residues possibly from derivatization agent and other breakdown components of the garlic matrix were found to easily contaminate the HP-5MS (5% diphenyl−95% dimethylpolysiloxane) column, and hence a further column heating step was required to purge the column after each run, which added to the overall run time.

Analysis of a series of individual thiol standards (10, 5, 2, 1, 05, 0.1, and 0.05 mg L^{-1}) produced calibration curves, which were averaged from triplicate analyses. The equations of the lines, R^2 values, and associated data obtained are given in Table 4. Detection limits were computed from GC background noise estimates ($3 \times SD/R^2$ value). Data suggest that both techniques hold promise and can be utilized in the determination of thiols from complex matrices, in this work, for garlic. Both techniques give comparable ranges of quantitation and linearity and detection limits. The advantage of derivatization in such techniques allows lower limits of detection, and, perhaps, offers a way to be able to elucidate particular minor components of a sample matrix for quantitation through peak enhancement and better chromatographic response. Use of the headspace method for delivery of the prederivatized needle or fiber to the sample minimizes the unwanted effects associated with in situ derivatization techniques and gave a clean chromatogram from which to analyze and extract the analyte of interest from a complicated sample matrix.

The garlic samples were prepared in two formats for analysis: (1) as a whole clove and (2) as a finely divided chopped clove. Nonderivatized thiophene gave a clean well-resolved peak, and hence this thiol component could be monitored in garlic samples (Tables 5 and 6 during certain evolution time analysis

Table 5. Analysis of Target Thiol Compounds from Garlic (Whole Clove) by Derivatized HS-SPME-GC-MS[a] and from Derivatized Exhaustive HS-NTD-GC-MS[b]

	compounds detected[c] (mg L^{-1}) after		
compound	6 h	12 h	18 h
HS-SPME-GC-MS[a]			
2-mercaptoethanol	0.48	0.29	−
1-propanethiol	0.19	0.13	0.32
1-butanethiol	0.19	−	<0.05
thiophene	X**	X**	−
sodium thiolmethoxide	0.99	<0.05	0.53
sodium ethanethiolate	<0.05	−	−
HS-NTD-GC-MS[b]			
2-mercaptoethanol	−	−	−
1-propanethiol	0.27	0.13	0.22
1-butanethiol	6.28	2.65	−
thiophene	X**	X**	X**
sodium thiolmethoxide	2.06	−	−
sodium ethanethiolate	<0.05	−	−

[a]$N = 3$ (analysis was carried out using SPME with a commercial 65 μm PDMS−DVB fiber). [b]$N = 3$ (analysis was carried out using the NTD with a 50:50 DVB−Carboxen mixed phase sorbent bed, with a needle phase stability of RSD ∼3% for over 100 extraction repetitions). [c]Estimated from calibration curves of individual derivatized thiols under identical sampling conditions; ±5.4%, $N = 5$ (Table 4). X**, identified peak but not quantified; −, not detected.

periods for the garlic compounds). For the analysis of chopped garlic in particular, the NTD technique showed better extraction efficiency of the thiol target analytes. As an example, 1-butanethiol was detected earlier and at greater concentration (6 h, 0.27 mg L^{-1}) than with SPME (12 h, 0.13 mg L^{-1}) (Table 6). Thiol assignments in the garlic (whole and chopped) were based on GC standard retention times and MS identification. Figure 5 shows a representative total ion chromatogram obtained by derivatized HS-SPME analysis for whole garlic

Table 6. Analysis of Target Thiol Compounds from Garlic (Chopped Clove) by Derivatized HS-SPME-GC-MS[a] and from Derivatized Exhaustive HS-NTD-GC-MS[b]

compound	compounds detected[c] (mg L^{-1}) after 6 h	12 h	18 h
HS-SPME-GC-MS[a]			
2-mercaptoethanol	–	0.29	–
1-propanethiol	9.9	7.64	0.64
1-butanethiol	–	0.13	–
thiophene	X**	X**	X**
sodium thiolmethoxide	6.63	3.53	2.90
sodium ethanethiolate	0.5	0.18	–
HS-NTD-GC-MS[b]			
2-mercaptoethanol	–	–	–
1-propanethiol	7.45	12.32	6.48
1-butanethiol	0.27	0.78	–
thiophene	X**	X**	–
sodium thiolmethoxide	4.76	1.58	0.61
sodium ethanethiolate	0.56	0.2	<0.05

[a]N = 3 (analysis was carried out using SPME with a commercial 65 μm PDMS–DVB fiber). [b]N = 3 (analysis was carried out using the NTD with a 50:50 DVB–Carboxen mixed phase sorbent bed, with a needle phase stability of RSD ~3% for over 100 extraction repetitions). [c]Estimated from standard addition plots of spiked target analyte garlic samples (10, 1, 0.5, and 0.05 mg L^{-1}) analyzed at the 6 h interval, ±5.4%, N = 5 and by comparison to calibration curves of individual derivatized thiols (Table 4). X**, identified peak but not quantified; –, not detected.

sample (a) when a fresh sample was taken, (b) when the sample was 6 h old, and (c) when the garlic sample was 18 h old. Similar results were obtained from NTD analyses. Derivative thiol peaks were identified by comparison to the studied thiol standard GC retention times and confirmed by MS identification. Estimates of amounts extracted for each of the derivatized thiol analytes for the techniques studied were carried out using a calibration curve (Table 4) obtained for a mixture of the four target analytes studied. Analysis (N = 5) for each of the standards and the individual thiol analyte concentration was calculated on the basis of their derivatized peak area counts. Also, thiols of concentrations (10,1, 0.5, and 0.05 mg L^{-1}) were spiked into the 2 g chopped garlic samples and analyzed at the 6 h time interval. A nonspiked 2 g chopped garlic sample was also analyzed at the 6 h time interval under identical conditions. Comparison of such data showed little deviation (<1.2%) from the calibration curve data, and resulting concentrations derived from these data are reported in Table 6. Spiking standards in whole garlic samples were not attempted. Estimates in Table 5 for thiol components from whole garlic for both techniques are then based solely upon comparison with calibration curve data.

As can be seen in Figure 5 and Tables 5 and 6, marked changes are seen over the three sampling time periods in the evolution of the chromatographed peaks, including the thiol peaks from both garlic samples. Volatile component concentrations can equilibrate at different times and temperatures[2] and vary tremendously in a sample over time. Hence, concentrations found for the individual thiol compounds at the 1, 6, 12, and 18 h samples are given as estimated. It is important that in the sampling foodstuffs or samples in which evolution is occurring, identically timed and temperature-controlled samples must be compared, to ensure that samples are under identical conditions, including instrumental and method optimization. Furthermore, such data may be able to be used to give insight as to the kinetic rate of evolution of components, rate constants, and partition ratio data for individual thiol components from foodstuffs.[25] Future kinetic investigation might give better insight as to whether this technique could give kinetic data that could be utilized in a wider scope, to follow the general evolution for a variety of components from natural foodstuffs.

In summary, the evolution of SPME and NTDs is driven by the need for a true one-step sampling process, whereby the sampling and extractions are reproducible and robust enough to enable reproducible sampling from complex matrices. The results presented here suggest that employment of these sampling and extraction methods may be useful in this regard, if control of the sampling/extracting conditions can be maintained. Both techniques offer attractive and comparative sampling and extraction methods and have demonstrated comparable detection, linearity, and ranges for limits of quantitation for selected thiol compounds. Coupling derivatization in-needle or in-fiber for NTD and SPME techniques (respectively) allows both techniques to be substantially pushed to enable lower detection limits and expands its function to target analytes not efficiently analyzed by typical SPME or NT techniques. This has been achieved without difficulties of derivatization agent or sample matrix contamination and thus avoids many complications associated with derivatization. As such, sample automation of run sequences is simplified as fewer automation steps are involved. SPME and NT have been successfully used to analyze thiol compounds in food samples. In addition, such techniques may provide an opportunity to study the kinetics of individual components from a variety of foodstuffs and complicated matrices, although further research is required to support this suggestion. Perhaps a drawback to the NTD is that it currently has to be homemade. Thus, time, access to a machine shop, and the learning curve to be able to master the technique and to handle and pack the small-diameter NTD in a consistent manner are required to prepare and to ensure reproducibility between one NTD and another. Such details are available.[24] Calibration of each NTD must be made after the device is built, although good reproducibility has been routinely achieved. Comparison between NTDs can be achieved by utilizing a target analyte standard to optimize the amount of analyte extracted, in this case, using a HS-in-needle derivatization-NTD-GC-MS method. On the basis of this approach, the coefficient of variation was reported to be <3% between the NTDyou feel better!s used. For alkanethiol determination, the NTD was not found to outperform the commercially available SPME PDMS fibers. However, for volatile and in-air sampling schemes, NTDs have shown a marked improvement in sensitivity and detection limit.[17,18] The small size of both the SPME fiber and the NTD makes such techniques desirable for on-site or quality control sampling. If the sorbent phase can be shown to act as a zero sink to the analyte of interest, then it is possible that negligible analyte loss would occur during transport. In this study, both devices behaved as a zero sink for garlic sample investigations, and this has allowed derivatization without analyte loss before transportation to desorption in the GC port.

AUTHOR INFORMATION

Corresponding Author

*Phone: +1 (709) 637-6200. Fax: +1 (709) 639-8125. E-mail: dparkins@grenfell.mun.ca.

Funding

We thank the Natural Science and Engineering Research Council of Canada (NSERC) and the Centre of Environmental Excellence, Corner Brook, NL, for funding.

Notes

The authors declare no competing financial interest.

REFERENCES

(1) Demirkol, O.; Adams, C.; Ercal, N. Biologically important thiols in various vegetables and fruits. *J. Agric. Food Chem.* **2004**, *52*, 8151−8154.

(2) Kim, N. Y.; Park, M. H.; Jang, E. Y.; Lee, J. Volatile distribution in garlic by solid phase microextraction (SPME) with different processing conditions. *Food Sci. Biotechnol.* **2011**, *20*, 775−782.

(3) Itakura, Y.; Ichikawa, M.; Mori, Y.; Okino, R.; Udayama, M.; Morita, T. How to distinguish garlic from the other *Allium* vegetables. *J. Nutr.* **2001**, *131*, 963S−967S.

(4) Gupta, N.; Porter, T. D. Garlic and garlic-derived compounds inhibit human squalene monooxygenase. *J. Nutr.* **2001**, *131*, 1662−1667.

(5) Stoll, A.; Seebeck, E. Specific constituents of garlic. *Sci. Pharm.* **1950**, *18*, 61−79.

(6) Rosenfeld, J. Chemical derivatization in analytical extractions. In *Handbook of Sample Preparation*; Pawliszyn, J., Lord, H. L., Eds.; Wiley: New York, 2010; Chapter 13, pp 225−245.

(7) Parkinson, D.-R. Analytical derivatization techniques. In *Comprehensive Sampling and Sample Preparation*; Lord, H. L., Pawliszyn, J., Eds.; Elsevier, Academic Press: Oxford, UK, 2012; Vol. 2 (Theory of Extraction Techniques), Chapter 26, pp 559−595.

(8) Shimada, K.; Mitamura, K. Derivatization of thiol-containing compounds. *J. Chromatogr., B* **1994**, *659*, 227−241.

(9) Parkinson, D.-R.; Dust, J. M. Overview of the current status of sediment chemical analysis: trends in analytical techniques. *Environ. Rev.* **2010**, *18*, 37−59.

(10) Salvador, A.; Moretton, C.; Piram, A.; Faure, R. On-line solid-phase extraction with on-support derivatization for high-sensitivity liquid chromatography tandem mass spectrometry of estrogens in influent/effluent of wastewater treatment plants. *J. Chromatogr., A* **2007**, *1145*, 102−109.

(11) Vesely, P.; Lusk, L.; Basarova, G.; Seabrooks, J.; Ryder, D. Analysis of aldehydes in beer using solid-phase microextraction with on-fiber derivatization and gas chromatography/mass spectrometry. *J. Agric. Food Chem.* **2003**, *51*, 6941−6944.

(12) Kullman, J. P.; Yu, T.; Chen, X.; Neal, R.; Ercal, N.; Armstrong, D. W. Biologically important thiols in various vegetables and fruits. *J. Liq. Chromatogr. Relat. Technol.* **2000**, *23*, 1941−1952.

(13) Wang, Q.; Chong, J. M.; Pawliszyn, J. Determination of thiol compounds by automated headspace solid-phase microextraction with in-fiber derivatization. *Flavour Fragrance J.* **2006**, *21*, 385−394.

(14) Parkinson, D.-R.; Warren, J. M.; Pawliszyn, J. Analysis of ergosterol for the detection of mold in soils by automated on-fiber derivatization headspace extraction-SPME-GC/MS. *Anal. Chim. Acta* **2010**, *2*, 181−187.

(15) Lord, H. L. Drug analysis by SPME. In *Handbook of Solid Phase Microextraction*; Pawliszyn, J., Ed.; Chemical Industry Press: Beijing, China, 2009; Chapter 10, pp 311−312.

(16) Pan, L.; Adams, M.; Pawliszyn, J. Determination of fatty acids using solid phase microextraction. *Anal. Chem.* **1995**, *67*, 4396−4430.

(17) Mieth, M.; Schubert, J. K.; Groger, T.; Sabel, B.; Kischkel, S.; Fuchs, P.; Hein, D.; Zimmermann, R.; Miekisch, W. Automated needle trap heart-cut GC/MS and needle trap comprehensive two-dimensional GC/TOF-MS for breath gas analysis in the clinical environment. *Anal. Chem.* **2010**, *82*, 2541−2551.

(18) Mieth, M.; Kischkel, S.; Schubert, J. K.; Hein, D.; Miekisch, W. Multibed needle trap devices for on site sampling and preconcentration of volatile breath biomarkers. *Anal. Chem.* **2009**, *81*, 5851−5857.

(19) Prikryl, P.; Kubinec, R.; Jurdakova, H.; Sevick, J.; Ostrovsky, I.; Sojak, L.; Berezkin, V. Comparison of needle concentrator with SPME for GC determination of benzene, toluene, ethylbenzene, and xylenes in aqueous samples. *Chromatographia* **2006**, *64*, 65−70.

(20) Eom, L.-Y.; Niri, V. H.; Pawliszyn, J. Development of a syringe pump assisted dynamic headspace sampling technique for needle trap device. *J. Chromatogr., A* **2008**, *1196−1197*, 10−14.

(21) Hang, S. S.; Yu, J. Z. Feasibility of collection and analysis of airborne carbonyls by on-sorbent derivatization and thermal desorption. *Anal. Chem.* **2002**, *74*, 1232−1240.

(22) Chen, Y.; Pawliszyn, J. Time-weighted average passive sampling with a solid-phase microextraction device. *J. Anal. Chem.* **2003**, *75*, 2004−2010.

(23) Stevens, R.; Stevens, L.; Price, N. C. The stabilities of various thiol compounds used in protein purifications. *Biochem. Educ.* **1983**, *11*, 70.

(24) Gong, Y.; Eom, L.-Y.; Lou, D.-W.; Hein, D.; Pawliszyn, J. Development and application of a needle trap device for time-weighted average diffusive sampling. *Anal. Chem.* **2008**, *80*, 7275−7282.

(25) Laidler, K. J. Analysis of kinetic results. In *Chemical Kinetics*, 3rd ed.; Harper and Row: New York, 1987; Chapter 2, pp 26−40.