Solid Phase Microextraction (SPME) on-fiber derivatization using a stable, portable, and reusable pentafluorophenylhydrazine (PFPH) standard gas generating vial

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ABSTRACT

Solid phase microextraction (SPME) on-fiber derivatization methods have facilitated the achievement of lower detection limits and targeted analysis of various substances that exhibit poor chromatographic behavior, thermal instability, or high reactivity while limiting the use of organic solvents. However, previously developed on-fiber derivatization methods have been hindered by poor loading reproducibility and standard lifetime due to derivatization reagent reactivity. In addition, this reactivity often results in these reagents demonstrating toxic effects, complicating handling and standard formulation. To address this a reusable standard gas generating vial containing pentafluorophenyl hydrazine (PFPH) has been developed. With this development SPME fibers can now be reproducibly loaded with derivatization reagent, from an easy to use and safe platform. Validation of the vial using C_4-C_9 linear aldehyde standards as target analytes demonstrated intra-batch vial reproducibility (2% RSD, n=4), along with PFPH headspace stability over a period of 11 weeks, facilitating reduced reagent consumption due to standard longevity. In addition, reproducibility of the derivatization reaction was observed over 1 week (RSD <9%) and the linear dynamic range was evaluated using headspace extractions from aqueous aldehyde solutions (R^2 >0.996, 10-200 ppb/v). Finally, the PFPH-generating vial was applied to the monitoring of volatile aldehydes generated during meat spoilage, and an on-site application using a portable GC-MS where the free and total concentration of formaldehyde was determined in car exhaust. To the best of our knowledge, the standard gas generating vial proposed in this work is the first documented device for the long term storage of a reusable headspace standards for a reactive, toxic and otherwise unstable derivatization reagent.

INTRODUCTION

Due to the broad range of organic compounds present in environmental and anthropogenic sample matrices, targeted analysis can be a difficult and often laborious task, requiring extensive and selective sample preparation. As a result, performing analysis of a particular class of compounds (e.g., aldehydes) requires the selection of an appropriate analytical method. Such targeted methods often consist of one or more analyte-specific steps that may impact different stages of the analytical process. Examples include the selective extraction of analytes by use of selective sorbents (e.g., molecular imprinted polymers)[1] or through derivatization. Derivatization is of specific interest, as it can aid in improving both chromatographic separation and detector sensitivity of a given analyte, while proving essential in the gas chromatographic (GC) analysis of compounds with poor thermal stability. Additionally, derivatization affords the opportunity for functionalities to be attached to certain analytes, such as halogens, which can then be targeted during instrumental analysis. The attachment of such functionalities then allows for the use of analytical instruments such as electron capture detectors (ECDs), which have been demonstrated to have remarkably low limits of detection. Additionally, selected ion monitoring mass spectrometry (SIM) for ions related to the derivatization reagent can also be employed, facilitating accelerated data processing.

Aldehydes and other carbonyl compounds are of particular environmental interest when considering their wide range of biogenic and anthropogenic sources.[2,3] Derivatization of carbonyl compounds can be accomplished in a variety of ways, and though not an exhaustive list, some applications include the following: a) microfluidic chips, where a derivatization agent solution and an air sample are mixed in a micro reactor;[4,5] b) impingers, which operate on the same general principle, effectively scrubbing the sample gas of analyte by passing it through a solution containing a derivatization reagent; or c) the use of conventional solid phase extraction (SPE), where a sorbent has been pre-loaded/impregnated with a derivatization reagent prior to sampling.[2,6] As an alternative, SPME can be applied towards derivatization through a process known as on-fiber derivatization, where the derivatizing agent is loaded onto the SPME fiber prior to, or after, the sample extraction. As SPME combines both sampling and sample preparation into a miniaturized, solvent-free format, it lends itself to greener sampling opportunities, and when coupled to portable instrumentation, rapid on-site sample analysis.[7–9] In order to fulfill the growing demand toward instrument portability and on-site analysis, it is imperative that techniques and methods that can be easily taken into the field are continuously

developed and improved to facilitate comprehensive and quantitative sampling. Though on-fiber derivatization for the sampling of carbonyl compounds is well documented in the literature,[10–25] as well as its various applications for ozone,[26] organometallic compounds,[27] chlorophenols in water,[28] primary amines in sewage,[29] and pharmaceuticals in meat products,[30] these methods have not been particularly portable. Specifically, the absence of a portable and reusable derivatization agent standard, which is required to load the fiber with an appropriate derivatization reagent prior to sampling, has impeded both the automation and the portability of such methods.

To address this need, a portable pentafluorophenyl hydrazine (PFPH) standard headspace (HS) generating vial has been developed based on the standard analyte generators previously described by Gomez-Rios *et al.*[31] and Grandy *et al.*[32] These vials operate under the principle of thermodynamic equilibrium between an analyte-spiked composite sorbent, comprised of polystyrene-divinylbenzene (PS-DVB) resin particles and silicon oil, and a gaseous headspace to generate a reproducible headspace concentration. These vials were shown to demonstrate remarkable reusability owing to the high affinity of the analyte for the composite sorbent, giving repeatable SPME fiber loadings over many extractions (n>200), and ensuring negligible depletion of the vial. Said vials are ideal for applications such as quality control and on-site/in-lab calibration. The currently presented gas generating vial application towards the storage of the highly reactive derivatization reagent PFPH demonstrates the first stable, portable, and reusable headspace standard of such a molecule. In this work, the PFPH generating vial was successfully coupled to the portable TRIDION-9 GC-toroidal ion trap mass spectrometer (TMS), such that on-site, on-fiber derivatization was used to quantify formaldehyde from car exhaust. In addition, the vial was coupled with a GCxGC-TOF/MS using SPME and applied towards the monitoring of meat spoilage over time, using aldehydes as markers of tissue decomposition.[33]

EXPERIMENTAL SECTION

Materials and reagents

Butanal, pentanal, hexanal, heptanal, octanal, nonanal, benzene, toluene, ethylbenzene, and xylene, along with PFPH and the 37 weight percent (wt. %) formaldehyde solution used were all purchased from Sigma-Aldrich (Mississauga, ON, Canada). Nano-pure water was obtained using a Barnstead/Thermodyne generating system (Dubuque, IA, USA). 65µm PDMS/DVB and 100µm PDMS SPME fibers with stableflex cores were purchased from Supelco (Bellefonte, PA, USA), while the operated Tenax/Carboxen (CAR) 1001/ CAR 1003 needle trap device (NTD) was provided by Torion

Technologies of PerkinElmer (American Fork, UT, USA). Ground beef samples were purchased from a local grocery store.

Standard gas generating vials were prepared as described by Grandy *et al.*[32] Aldehydes C_4 to C_9 were spiked into silicon oil (Kurt J. Lesker Company, Toronto, ON, Canada) at 0.005, 0.007, 0.009, 0.010, 0.013 and 0.015 wt. %, respectively. Similarly, instrumental quality control vials were manufactured by spiking benzene, toluene, ethylbenzene, and *o*-xylene into silicon oil at 0.005, 0.007, 0.010, 0.010 wt. %, respectively. Finally, the vials containing PFPH had a silicon oil loading of 1.19 wt. %, ensuring significant fiber loadings while using minimal exposure times. A detailed description of the aqueous aldehyde standards is provided in Supplementary Information (Section 1).

In order to ensure constant vial temperature, allowing for reproducible SPME fiber loadings, vials were heated in block-heaters developed and fabricated at the University of Waterloo Science Shop. Each block-heater consisted of an electrical heater connected to a thermocouple, and provided temperature accuracy of ± 0.1ºC. As shown in Figure S.1, a portable battery-operated block-heater was also assembled for on-site use of the analyte generating vials.

Instrumentation

All on-site analyses were performed using a TRIDION-9 portable GC-TMS (Torion Technologies of PerkinElmer, American Fork, UT, USA). Chromatographic separations were performed using a low thermal mass MXT-5 (5 m x 0.1 mm x 0.4 µm) Siltek ® (Restek, MA, USA) treated stainless steel column, with helium as carrier gas at a flow rate of approximately 0.3 mL min^{-1}. The column temperature was initially held at 50 ºC for 10 seconds, and then increased to 250 °C at a rate of 1.5 $^{o}C\ s^{-1}$ and held there for 15 seconds. Desorption of the DVB/PDMS fiber and tri-bed NTD was carried out under splitless conditions for 10 s at a temperature of 270 ºC, followed by an opening of the 10:1split valve for an additional 30 seconds. Ionization was performed using an electron-impact ion-source (electron ionization), and the toroidal ion trap operated in a customized scan mode of 43-400 m/z.

Stability studies and vial validation experiments were conducted using an Agilent 5890 GC-FID (Santa Clara, CA, USA). Chromatographic separations were performed using an Agilent DB-5 column (30m x 0.250mm x 0.25 µm) (Santa Clara, CA, USA). The column temperature was initially held at 42 °C for 1 minute, then increased to 250 °C at a rate of 20 $^{o}C\ min^{-1}$, and held there for 3 minutes. Desorption of the DVB/PDMS fiber was carried out for 2 minutes at a temperature of 260 ºC in splitless mode.

Application of the PFPH standard gas generating vial to the semi-quantitative analysis of aldehydes as markers for meat spoilage was conducted using a GCxGC-TOF/MS Pegasus 4D (LECO Corp.,

St Joseph, MI, USA). The chromatographic system consisted of an Agilent 6890 GC oven containing a secondary oven and a quad-jet modulator, consisting of two hot-air jets and two cold nitrogen jets created by liquid nitrogen. The column configuration consisted of a Rtx®-5SilMS (30m x 0.25 mm x 0.25 µm) (Restek Corp., Bellefonte, PA, USA) capillary column in the first dimension (1D), and a BP-20 (1m x 0.1mm x 0.1 µm) (SGE Analytical Science, Trajan Scientific Australia Pty Ltd) in the second dimension (2D), connected by a SilTite® µ-Union (SGE Analytical Science, Trajan Scientific Australia Pty Ltd). A modulation period of 4 sec was used, with a hot pulse duration of 0.6 sec, and a cold pulse time of 1.4 sec. The desorption of analytes from the SPME coating was performed in splitless mode at 270 °C for 10 min, using ultra high purity helium as carrier gas with a constant flow rate of 1.5 ml/min. The primary oven temperature was initially held at 50 °C for 1 minute, followed by a temperature ramp of 20 °C/min until a final temperature of 250 °C was reached, then subsequently maintained for 30 seconds. The offset for the secondary oven temperature was set at 10°C above the primary oven temperature. The modulator offset was set at +15°C. The transfer line and ion source temperatures were set at 250°C and 200°C, respectively. Electron impact ionization was performed at an energy output of 70 eV. A solvent delay of 60 sec was employed. Mass range scanning was set at 35-600 m/z.

Safety considerations

PFPH is acutely toxic and can cause serious eye irritation. As a result, special care was taken at all times while handling this material. Precautions included the use of Nitrile gloves, conventional laboratory coats, and a full face mask/respirator during handling. With the exception of the analytical weighing of the pure PFPH standards, all formulation was conducted inside a fume hood. However, once encased inside a standard gas generating vial, the standard was considered safe to handle.

Extraction conditions from the standard analyte generating vials

The standard gas generating vials containing PFPH and the BTEX QC were maintained at 35°C throughout the experimentation, while vials containing linear aldehydes C_4 through C_9 were maintained at 50°C in order to provide a higher headspace concentration of the heavier aldehydes. While not in use, vials were stored in a low light environment at room temperature (20 ± 1 °C). Prior to use, all vials were allowed to equilibrate to their experimental temperature for no less than one hour prior to sampling. Similarly, after each extraction, vials were left to re-equilibrate for at least 10 minutes to ensure re-

equilibrium was achieved between the headspace and the sorbent phase.[32] All SPME extractions were performed using DVB/PDMS fibers, as they have been previously shown to demonstrate a strong affinity for PFPH.[18] Extraction times from each vial varied between 10 seconds and 10 minutes, depending on the parameter under study. One-minute quality control extractions were also performed from a BTEX-generating vial throughout the experiments, using a dedicated DVB/PDMS SPME fiber to monitor the stability of the analytical instrumentation throughout the study.

Methodology of on-fiber derivatization extractions

To perform the aforementioned on-fiber derivatization using the proposed standard gas generating vial, the following steps were implemented; first, the SPME fiber was pre-loaded with PFPH by performing a headspace extraction from the PFPH-generating vial. Subsequently, the fiber was removed from the vial, and then exposed to the sample matrix, where carbonyl compounds underwent a dehydration reaction with the PFPH. The reaction itself is described in Figure S2, along with a brief description of the formation of the major ions observed in MS analysis (Section S2). Similarly, in Figure S3, an example can be found of an obtained mass spectra. Finally, the fiber was thermally desorbed in the GC injection port for analytical separation and detection.

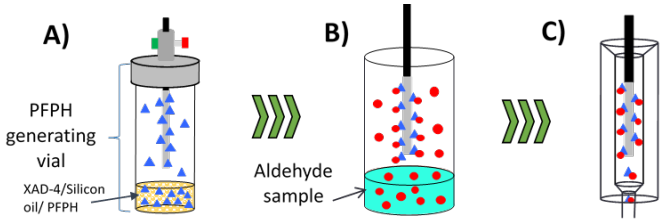

Figure 1 On-fiber derivatization procedure employing the new PFPH-generating vial. **A)** PFPH loading of SPME fiber, **B)** PFPH-loaded fiber exposed to gaseous aldehydes, resulting in derivatization, **C)** thermal desorption of SPME fiber on the GC-injection port.

Long term storage stability of the PFPH generating vial

In order to ensure representative stability data, eight PFPH vials were formulated. Of these vials, four were capped using Mininert® caps, while the remaining four were capped using conventional screw-on septa caps.[31] Headspace volumes were verified to be the same in both formats. The Mininert® cap was selected due to its capability to provide a better seal than that of a septa cap after multiple punctures. Given that an imperfect seal may allow for the introduction of atmospheric contamination of

the headspace, resulting in eventual PFPH degradation or loss, the quality of the seal is considered a critical parameter. One vial of each cap type was placed in a fridge for storage at 5°C, while the remaining six vials were placed in a dark cupboard at room temperature. This one done so that any differences in terms of storage temperature could be observed. Of the six vials stored in a dark cupboard, two of each type were sampled in triplicate at days 3, 7, 14, 30, and 77 after vial formulation. The remaining two vials, and the two stored in the refrigerator, were only sampled on days 30 and 77. This step was employed so as to observe whether any discrepancies could be observed between the vials that had been sampled previously and those that had not. All extractions, consisting of 10 minute extractions conducted from the headspace of the PFPH gas generating vial, were performed under the same conditions. The GC-FID used for this study was calibrated for PFPH response by liquid injection, using standards prepared in hexane. Throughout the 77 day experiment, QC runs were performed in order to monitor instrumental variabilities. All data was quality control adjusted to the day of calibration, although the GC-FID used for the experiments remained within two standard deviations of the population mean of each QC standard for the duration of the experiment. A detailed outline of this experiment can be seen in Figure S4 of the Supplementary Information.

Extraction of Aldehyde Spoilage Biomarkers from Ground Beef

Ground beef samples were prepared by weighing 5 g of raw medium ground beef into 20 ml amber glass headspace vials, and stored at room temperature. In order to increase the headspace concentration of semi-volatile and volatile sample constituents, all samples were equilibrated for 1 hour at 35^{o}C prior to extraction. Following a 5-minute pre-loading of the derivatization agent, static headspace extractions of the beef samples were carried out for 30 minutes at 35^{O}C. In order to monitor the production of aldehydes during the meat spoilage process, extractions were performed in duplicate, with and without derivatization, on the day of vial preparation and 1, 5, and 7 days after sample preparation. All extractions were performed from previously un-sampled vials to ensure cross-contamination from the PFPH did not occur.

Onsite Analysis of Formaldehyde from Car Engine Exhaust

On-site samplings of formaldehyde from car engine exhaust were performed by first loading the derivatization agent onto the SPME fiber through a 10 minute extraction from the headspace of the PFPH-generating vial. While, 15mL of the PFPH vial headspace was drawn onto the sorbent bed of the needle trap device. In order to facilitate this volumetric NTD extraction from the vial, a blank 22 gauge

needle was also inserted through the vial septum to allow the headspace pressure to remain constant. Then, the PFPH-loaded sampling devices were positioned in the center of the vehicle exhaust. For NTD samplings,[34] 10mL of sample was drawn from both hot and cold engine exhaust, while SPME fiber extractions were performed for 60s and 30s, respectively. Prior to cold exhaust extractions, the catalytic converter was first verified to be cool to the touch. Next, the engine was allowed to idle for 30s after ignition, followed by sampling. For hot exhaust extractions, the vehicle was allowed to idle continuously, with sampling starting at 30 minutes after ignition. To ensure stable instrument response, quality control extractions were performed on-site and during calibration.

As it is infeasible to perform calibration by liquid injection on portable GC-MS instrumentation due to the limited column capacity, a novel, NTD-based calibration technique was employed. To achieve this, a NTD was first pre-loaded with PFPH by successively extracting 10 mL from two separate septa-capped PFPH generating vials. Volumes of 0.1, 0.5, 1, 2, 4, and 6mL were then drawn from the headspace above 10mL of 0.04wt% aqueous formaldehyde in a 20mL headspace vial under 1500rpm magnetic agitation. Extractions were verified to exhibit negligible depletion by performing a subsequent extraction from the same vial. The temperature dependent Henry's Law constant, as determined by Seyfioglu et al.,[35] was then used to calculate the concentration of formaldehyde above such a solution, which was found to be 2.97 ng/mL. The obtained concentration was then used to determine the amount extracted by the needle trap with the use of Equation 1, where n is the amount extracted, C is the headspace concentration, and V is the sample volume.

$$Equation\ 1) \qquad n = C\,V$$

Each point was analyzed in triplicate from freshly spiked formaldehyde solutions. The resulting response versus extracted mass (nanograms) calibration plot was observed to demonstrate a strong correlation (R^2=0.9988, FigureS.11). However, the y-intercept for the line of best fit (19,816) was of similar magnitude to the response of the NTD extractions from hot car exhaust. As a result, the calibration plot was weighed by $1/x^2$, resulting in the relationship y = 51305x + 4145, which was then used to calibrate the SPME fiber and NTD on-site samplings. Once the response relationship had been determined, further calibrations of the SPME extractions were performed using diffusion-based calibration, as described by Koziel et al.[36]

RESULTS AND DISCUSSION

PFPH vial long-term stability

Considering that current methods used in the generation of gaseous PFPH standards fail to provide a reusable headspace standard for extended periods of time, a requirement for on-site applications and standard storage, the long-term stability of PFPH within the standard gas generating vial was determined. As exemplified in Figure 2, the vials were found to be stable over a 77-day period. Having no vial which was sampled on days 3, 7 14 and 77 exceeding a %RSD over 9% (Figure S.5, Table S.2). Similarly, storage conditions, sampling history, and cap type were found to have no impact on absolute fiber loadings as shown in Figure S.6. Moreover, good intra-batch reproducibility for PFPH fiber loadings was observed for vials tested on days 3, 7, 14, 30, and 77, providing %RSDs of 2, 5, 5, 5, and 9, respectively, when the average of that day was considered, as presented in Tables S.1 and S.2. Finally, and most impressively, when all extractions from all vials over the entirety of the stability study were considered, the average amount of PFPH loaded onto the DVB/PDMS SPME fibers was 2729 +/- 196 ng, with a %RSD of 7%.

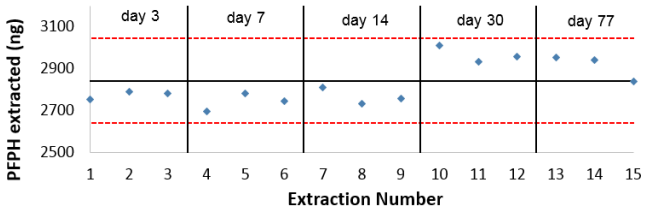

Figure 2 Amount of PFPH extracted onto a PDMS/DVB fiber over 77 days C from septa vial #3, using a 10 minute sampling time at 35°. Data was normalized from daily quality control experiments.

Reproducibility of the derivatization reaction

As PFPH fiber loadings were shown to be reproducible, we would expect that derivatization reaction itself should also be reproducible. In order to confirm this, the reproducibility of the derivatization reaction was examined over a 7-day period. This validation was accomplished by using an aldehyde (C_4-C_9) standard gas generating vial in addition to the PFPH vial, with samplings being performed in triplicate in two-day intervals. Pre-loading of the SPME fiber with PFPH was performed for 5 minutes, followed by a 1 minute exposure to the aldehyde-generating vial headspace.

As demonstrated in Figure S.7, the derivatization reaction was found to be very reproducible over the 7-day test period, with the response of the derivatized aldehydes remaining constant, as

indicated by %RSD values of 4, 4, 4, 5, 5, and 9 for derivatized butanal, pentanal, hexanal, heptanal, octanal, and nonanal, respectively. The upward trend in %RSD with respect to molecular weight was thought to be a result of decreasing signals for these heavier aldehydes. This decrease in signal was likely due to a decrease in the headspace concentration of the heavier aldehydes, as they possess a higher vial sorbent affinity and diffuse slower through the boundary layer of the fiber.[7]

Derivatization products extraction time profile

Although the derivatization reaction exhibited a good degree of reproducibility, even when performed repeatedly over 7 days, it was also important to ensure that the amount of derivatized product on the fiber could be predicted regardless of extraction time. Ideally, it would be expected that as long as some residual PFPH remains on the fiber, aldehyde extraction should remain linear with a slope dependent on that aldehydes rate of diffusion across the fibers boundary layer. Hence, a derivatization reaction time profile was conducted in order to confirm the linear uptake of the derivatization reaction products associated with the aldehydes studied. Using the aldehyde standard gas generating vial described prior, extraction times ranging from 10 seconds to 1 minute were conducted, while maintaining constant PFPH extraction conditions at 35^{o}C for 5 minutes.

As shown in Figure S.8, all derivatized aldehydes exhibited a linear relationship between analyte response and aldehyde extraction time over the sampling interval tested. As the y-intercepts of some compounds are well above zero, two linear regions of analyte uptake are hypothesized to be present, though employing a sampling time of less than 10 seconds is not often practical.

Aldehyde concentration effect on response

Due to the wide range of aldehyde concentrations in environmental and anthropogenic matrices, it is imperative that reaction response is understood over a broad range of aldehyde concentrations. Furthermore, it would be advantageous if this response would exhibit a linear relationship, such that quantitation can be easily performed. In order to investigate whether the method demonstrates a linear response with respect to aldehyde concentration, samples containing 10ppb to 200ppb of linear aldehydes C_4 through C_9 were formulated in aqueous solution. Prior to sample extraction, the SPME fiber was pre-loaded with PFPH for 5 minutes, followed by a 1 minute exposure to the headspace of the aqueous standard. Each extraction was performed in triplicate.

As clearly demonstrated in Figure 3, which shows the calibration plot for derivatized butanal, octanal, and nonanal, the response of the proposed method demonstrated excellent linear correlation with sample concentration. Furthermore, the results for derivatized pentanal, hexanal, and heptanal, presented in Figure S.9, also supported this conclusion. No residual unreacted aldehydes were observed in any chromatogram throughout these concentration studies, thus confirming that the reaction between PFPH and the tested aldehydes is equally quantitative at room temperature as it was at 50°C (aldehyde generation vial temperature).

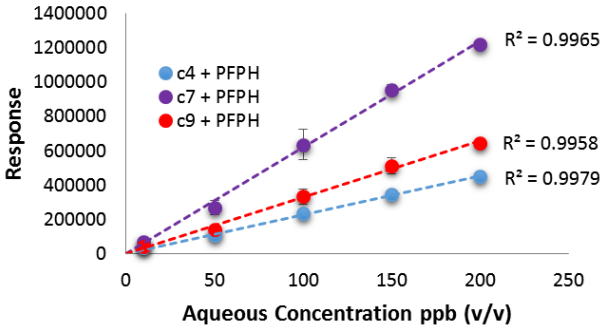

Figure 3: Demonstration of linear on-fiber derivatization response of butanal, heptanal, and nonanal for SPME headspace extractions above aqueous solutions ranging from 10-200ppb (v/v) at room temperature, using a 1 minute extraction.

Observation of volatile aldehydes from meat using GCxGC-TOF

With confirmation of a reliable method, the versatility of the PFPH-generating vial was demonstrated by its application to the semi-quantitative analysis of aldehydes as markers for meat spoilage.[33] A range of aldehydes have been linked to the breakdown of fatty acids during the decomposition of meat products, particularly in beef and pork, and have been identified as contributing factors in the off-flavor and odor associated with spoiled meat.[37] A sample of medium ground beef, advertised as fresh, was obtained from a local grocery store. Identification of the derivatized aldehydes in the analyzed meat samples was performed by comparison of the retention times and mass spectra with those of derivatized standards of aldehydes (C_4-C_9). Figure 4 shows the bi-dimensional chromatograms obtained for aldehyde standards extracted with and without derivatization (a,b), and for extractions carried out on meat samples on the day of preparation (day 0) with and without

derivatization (c,d). In addition, the isomerization of the derivatization reaction product was observed, and could be resolved in two dimensions.

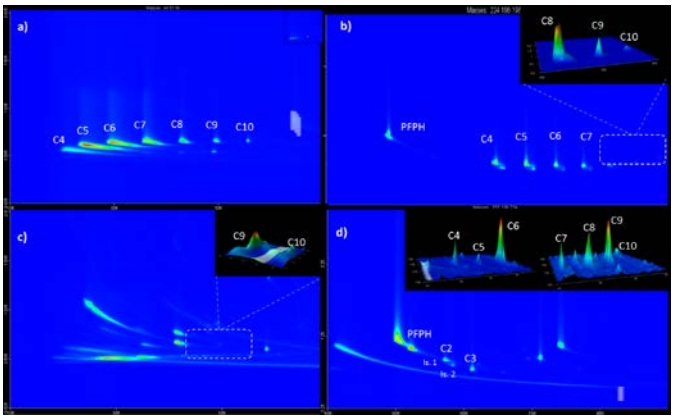

Figure 4 GCxGC-ToF/MS chromatograms of (a) underivatized and (b) derivatized C_4-C_{10} aldehydes standards, and (c) underivatized and (d) derivatized aldehydes sampled from the headspace of freshly prepared ground beef meat samples.

The response of volatile aldehydes from the meat samples was generally observed to increase over time, though reproducibility was low between duplicate samples. This was likely a result of varying degrees of microbial activity in each sample, in part due to inhomogeneity of the meat sample.[37] Though ground beef was used, further homogenization would have likely provided more reproducible results. However, as a proof of concept, the application of the PFPH standard gas generating vial to the analysis of food products was a success. In particular, the sampling performed without derivatization only resulted in the detection of C7 through C10 aldehydes with very poor sensitivity, whereas derivatization allowed for the detection of a much broader range of aldehydes (C2 through C10).

In order to further test the applicability of the on-fiber derivatization approach, a 100µm PDMS SPME fiber coating was also applied to the analysis of aldehydes from spoiled meat. This is particularly important, as PDMS coatings are more robust, and have been applied to both ex vivo and in vivo samplings from tissues,[38] minimizing the occurrence of possible artifacts associated with the extraction process.[39] Due to the hydrophobic nature of the PDMS polymer, successful extraction of underivatized and short-chain aldehydes could prove to be difficult due to their polarity and relatively low concentrations. Consequently, the risk of losing important chemical information when sampling complex matrices with PDMS under underivatized conditions is high. By repeating the derivatization protocol with a PDMS 100µm SPME fiber, C_2 and C_5-C_9 derivatized aldehydes were observed, though lower responses were observed than those obtained for the DVB/PDMS coating. Considering what has been

stated, it is evident that the PFPH standard gas generating vial can be implemented in the monitoring of meat spoilage processes. However, the optimization of such a method is not within the scope of this work.

Application for on-site quantitation of formaldehyde in car exhaust

As a final proof of concept, the portability of the PFPH-generating vial was confirmed by coupling the method to hand-portable GC-MS instrumentation for the quantitative on-site determination of formaldehyde from car exhaust. In order to compare the free and total formaldehyde concentrations before and after heating of the catalytic convertor, both needle trap and SPME extractions were performed in triplicate from cold and hot car exhaust. These sampling methods, namely SPME and NTD, were decided upon for use in view that NTDs are able to extract both free and particulate-bound analytes in an exhaustive manner,[34] while SPME fibers are only sensitive to the gaseous, unbound analyte fraction. This allows the analyst to compare the free and total analyte concentrations by SPME and NTD, respectively, so as to characterize the fraction of particulate binding of a given analyte, in this case formaldehyde. After extraction, both extraction devices were analyzed on-site using a TRIDION-9 portable GC-TMS, where derivatized formaldehyde was successfully identified by its derivatization reaction product molecular ion and fragmentation pattern.

For the needle trap extractions, the concentration of formaldehyde found in the car exhaust was easily determined by use of Equation 1. However, as SPME is an open-bed equilibrium-based extraction method, a diffusion-based calibration method was required.[36] The specifics of the employed diffusion-based calibration can be found in the Supplementary information (Section 3).

Extraction Condition	Exhaust Temperature (°C)	Sampling Device	Formaldehyde (ng/mL-exhaust)
Pre-catalytic convertor ignition	16.3	NTD	0.37 ± 0.02
		Fiber	0.18 ± 0.02
Post-catalytic convertor ignition	45.7	NTD	0.042 ± 0.003
		Fiber	0.035 ± 0.005

Table 1 On-site determination of free and total concentrations of formaldehyde from car exhaust pre- and post-catalytic convertor ignition, using a 10 minute PFPH loading time for SPME extractions and a 15mL sample volume from a PFPH generating vial at 35°C.

As can be seen in Table 1, no significant differences were observed between the concentrations of formaldehyde determined using the SPME fiber or NTD from the hot car exhaust samples, though

differences were observed in the cold car exhaust samples. With this in mind, it is possible to conclude that the vast majority of formaldehyde generated and subsequently vented via this particular exhaust system was in the gaseous fraction when the vehicle was running at its operational temperature. On the other hand, the results obtained for the cold car exhaust extractions indicate significant binding of formaldehyde to the particulate matter and aerosols generated and subsequently released by the vehicle immediately after engine ignition. Although we would typically expect the highly volatile formaldehyde molecule to preferentially move to the free, gaseous fraction of the sample at ambient temperatures, the presence of water laden aerosol particles may have greatly increased the particulate bound concentration as formaldehyde would tend towards dissolution into the aqueous portion of the aerosol particle. This again highlights the strength of using both SPME and NTD to fully characterize the speciation of analytes in real systems.[34] Furthermore, another interesting observation could be made with regards to the effectiveness of the catalytic convertor, as indicated by the reduction of total formaldehyde concentration by a factor of approximately 10 times when hot and cold car exhaust are compared.

Most importantly, this experiment may very well outline the first instance where quantitative analysis of formaldehyde was successfully performed entirely on-site with GC-MS instrumentation. Moreover, it shows just how portable SPME methods can be; amongst its many advantages, SPME allows for the on-site performance of repeated derivatizations of a target analyte from real samples when coupled to portable instrumentation.

CONCLUSION

A novel standard gas generating vial suitable for SPME on-fiber derivatization of aldehydes has been developed. The application of this technology results in the use of no organic solvent while also reducing the consumption of a toxic and highly reactive derivatization regent, due to the reusability and stability of the compound in the vial developed. In addition, the PFPH generating vial was successfully shown to provide a means by which to perform derivatization from food matrices facilitating the targeted analysis of carbonyl compounds from a complex sample matrix. The PFPH generating vial was then applied to the completely on-site determination of formaldehyde from car exhaust, owing to the portable nature of the standard gas generating vial. The application of the in-vial standard gas generator for derivatization agents is expected to be expanded into a range of fields including clinical, food, environmental and forensic analysis. Once fully realized, on-site sample derivatization and/or analysis

owes itself to exciting new applications of sampling, particularly for analytes which may not be stable under storage or transport conditions to the laboratory.

ASSOCIATED CONTENT

Supplementary Information is available online at DOI:

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CONFLICT OF INTEREST DISCLOSURE

The authors of this manuscript herein declare that although we have received financial support from Torion Technologies of Perkin Elmer Co. we maintain our independence as a 3rd party academic body resulting in an un-biased representation of the results with no competing conflict of interest, financial or otherwise.

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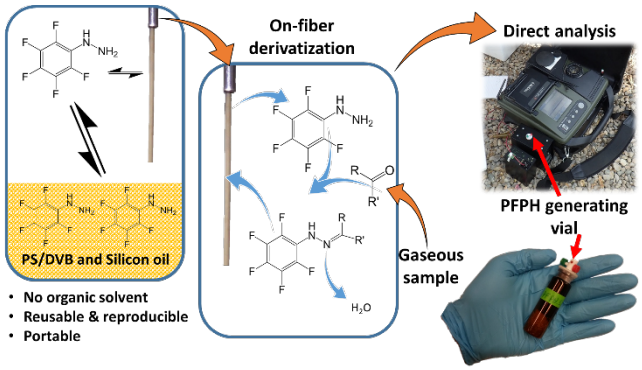

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