HHS Public Access

Author manuscript

Anal Chem. Author manuscript; available in PMC 2023 September 26.

Published in final edited form as:

Anal Chem. 2023 March 07; 95(9): 4344–4352. doi:10.1021/acs.analchem.2c04604.

Analysis of a broad range of carbonyl metabolites in exhaled breath by UHPLC-MS

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Abstract

Analysis of volatile organic compounds (VOCs) in exhaled breath has shown great potential for disease detection including lung cancer, infectious respiratory diseases, and chronic obstructive pulmonary disease. Although many breath sample collection and analytical methods have been developed for breath analysis, analysis of metabolic VOCs in exhaled breath is still a challenge for clinical application. Many carbonyl compounds in exhaled breath are related to metabolic processes of diseases. This work reports a method of ultra-high performance liquid chromatography coupled with high resolution mass spectrometry (UHPLC-MS) for analysis of a broad range of carbonyl metabolites in exhaled breath. Carbonyl compounds in exhaled breath were captured by a fabricated silicon microreactor with a micropillar array coated with 2-(aminooxy)ethyl-*N,N,N*-trimethylammonium (ATM) triflate. A total of six subgroups consisting of saturated aldehydes and ketones, hydroxy-aldehydes, and hydroxy-ketones, unsaturated 2-alkenals, and 4-hydroxy-2-alkenals were identified in exhaled breath. The combination of a silicon microreactor for selective capture of carbonyl compounds with UHPLC-MS analysis may provide a quantitative method for analysis of carbonyls to identify disease markers in exhaled breath.

Graphical Abstract

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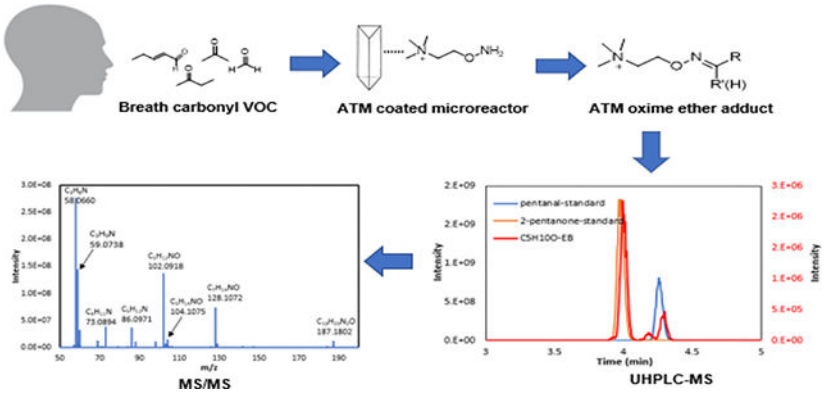
Author Contributions: All authors have given approval to the final version of the manuscript.

The authors declare no competing financial interest.

ASSOCIATED CONTENT

Supporting Information

ATM adduct calibration curves, microreactor flow rate effect, fragmentation scheme, UHPLC chromatogram, and MS^2 spectra for standards and exhaled breath samples. The Supporting Information is available free of charge on the ACS Publications website.



Breath analysis has received considerable interest because of its applications in noninvasive diagnosis of diseases.[1–5] Carbonyl compounds have been investigated because elevated levels of these compounds in exhaled breath are considered as potential biomarkers for many types of diseases.[6–10] Some carbonyl compounds, such as 2-butanone, hydroxy-acetaldehyde, 3-hydroxy-2-butanone, 2-pentanal, 2-hexanal, 4-hydroxy-2-hexenal (4-HHE), and 4-hydroxy-2-nonenal (4-HNE), are reported to have elevated levels in exhaled breath of lung cancer patients.[8–12] Malondialdehyde (MDA) and 4-HNE in breath have been identified as biomarkers of asthma, chronic obstructive pulmonary disease (COPD), cardiovascular disease, and bronchiectasis induced by oxidative stress and inflammation.[13–15] MDA and 4-HNE were also reported in several biological matrices including exhaled breath condensate,[16] tissue,[17] urine,[18] serum,[19] and bronchoalveolar lavage fluid.[19] An elevated level of acrolein (propenal) has been found in the brain of Alzheimer's disease subjects.[20] Therefore, the measurement of carbonyl compounds in biofluids is of great interest for disease detection and the study of disease etiology.

Among all the developed methods for analysis of volatile organic compounds (VOCs) in exhaled breath, gas chromatography-mass spectrometry (GC-MS) is one of the most widely employed techniques.[21–23] It allows separation and identification of VOCs from the breath matrix. However, GC-MS requires sample pre-treatment (pre-concentration and moisture removal), which leads to losses of analytes and contamination issues. GC-MS is also not suitable for real-time measurement.[24] Other methods have been used for real-time measurements without sample processing, such as selected ion flow tube-mass spectrometry (SIFT-MS),[25] proton transfer reaction-mass spectrometry (PTR-MS),[26] ion mobility spectrometry (IMS)[27] as well as electronic nose or gas sensor arrays.[28] Although these techniques can be applied for real-time analysis and show good sensitivity, none have the chromatographic separation or spectral libraries for comparing results when compound identification is involved.

To focus on analysis of carbonyl compounds in exhaled breath for clinical applications, this work reports a new approach that combines a carbonyl trapping agent along with a microfabricated silicon microreactor to capture carbonyl compounds via oximation reactions with analysis using ultra high-performance liquid chromatography-mass spectrometry (UHPLC-MS) for quantitative measurements. Previously, we developed 2-(aminooxy)ethyl-*N,N,N*-trimethylammonium salts (ATM) to capture volatile aldehydes and ketones using

a silicon-based microreactor followed by sample analysis using high resolution Fourier transform-ion-cyclotron resonance (FT-ICR) mass spectrometry (MS) without separation capacity.[29, 30] By developing the present analytical platform technology, a broad range of carbonyl compounds including two subgroups of unsaturated 2-alkenals, and four subgroups of saturated ketones and aldehydes have been separated and identified in exhaled breath samples and their concentration ranges were determined. The subgroups of hydroxy-aldehydes and hydroxy-ketones are for the first time identified and measured. Although unsaturated 2-alkenals, 4-hydroxy-2-alkenals and 4-hydroxy-2,6-alkadienals have been reported in exhaled breath condensates, there have been no concentration ranges reported for these compounds and no unsaturated aldehydes with less than 6 carbon atoms have been previously reported.[31] The present method enables detection of unsaturated aldehydes from C3 to C10 and unsaturated hydroxy-aldehydes from C3 to C9 in exhaled breath. A quantitative method for analysis of carbonyl compounds in exhaled breath can be used to identify disease biomarkers. Furthermore, the detection of these carbonyl compounds also provides evidence for metabolic and mechanistic studies of production of a large number of carbonyl compounds in exhaled breath from the oxidation of unsaturated fatty acids.[32]

EXPERIMENTAL SECTION

Material and methods

All reagents and solvents, including saturated aldehydes, ketones, hydroxy-ketones, hydroxy-aldehydes (except for 2-hydroxybutanal and 2-hydroxyheptanal, see supporting information for synthesis[33, 34]), acrolein (>99.5%), crotonaldehyde, 2-pentenal, 2-hexenal, 2-heptenal (>99.5%, mixture of cis and trans) were purchased from Sigma-Aldrich. 4-HHE and 4-HNE were purchased from Cayman. Water, acetonitrile, methanol, and formic acid of LC-MS grade were purchased from Fisher Scientific. Tedlar bags with a volume of 1 liter were also purchased from Sigma-Aldrich. 2-(Aminooxy)ethyl-*N,N,N*-trimethylammonium (ATM) triflate was synthesized from ATM iodide[35] using a counterion exchange protocol.[36] Eight ATM-carbonyl adduct standards were synthesized and purified according to the procedure described in Supporting Information (SI) for preparing calibration curves in Figure S1. Other adduct standards were prepared by mixing ATM with single carbonyl compounds with a molar ratio of 1.2:1 to ensure complete reaction of carbonyl compounds to obtain the retention times and MS^2 data of UHPLC-MS.

Silicon microreactors

The silicon microreactors were fabricated from 4-inch silicon wafers using standard microelectromechanical systems (MEMS) fabrication techniques. Details of the microreactor design and fabrication and characterization have been published elsewhere.[29, 30] The microreactor has thousands of equilateral triangular micropillars in an area of 21 mm in length and 7 mm in width and the surface of the micropillars were oxidized by wet (a mixture of H_2O and O_2) thermal oxidation. The triangular micropillars have a 50 μm lateral length and 400 μm in height, and the distance between the two closest pillars are 10 μm. Figure 1 (a) shows the size comparison of the microreactor with a dime coin, and (b) the SEM micrograph of the micropillars. The inlet and outlet of the microreactor were fitted with 350 μm O.D. and 250 μm I.D. deactivated fused silica tubes

using a silica-based bonding agent. The total empty volume of the microreactor is about 30 μL. The surface of the channels and micropillars were functionalized with ATM by infusing ATM in methanol solution (1.5 μmol, 30 μL) into the microreactor from one connection port followed by evaporation of the solvent under vacuum oven at 50 °C. Figure 1 (c) shows the schematic illustration of ATM cations adsorbed on the surfaces of micropillars for oximation with ketones and aldehydes.

Collection of exhaled breath samples

After approval by the Internal Review Board at the University of Louisville and obtained written informed consent, exhaled breath (EB) samples were collected from 20 healthy subjects using Tedlar bags. The Tedlar bags were cleaned with high purity nitrogen before each use to reduce background contamination. A 1L Tedlar bag was connected with a Teflon tube as mouthpiece. Subjects directly blew through the mouthpiece to fill the 1L Tedlar bag in one exhaled breath. A mixture of tidal and alveolar breath was collected. After collection, the gaseous breath in the Tedlar bag was evacuated through the microreactor at a flow rate of 7 mL/min. The setup for the evacuation to capture carbonyl VOCs requires a vacuum pump to pull gaseous breath samples from the Tedlar bag through the ATM-coated micropillar array in the microreactor.[29, 30]

UHPLC-MS analysis of carbonyl adducts

After the Tedlar bag was completely evacuated, ATM-carbonyl adducts in the microreactor were eluted with 200 μL of methanol from the microreactors. To the eluted sample 5×10^{-9} mol of ATM-acetone-d6 adduct was added as an internal reference (IR). Then the sample was diluted ten times with water for analysis. A Thermo Scientific ultra-high performance liquid chromatography-mass spectrometry (UHPLC-MS) system equipped with an automatic sampler, a Vanquish UHPLC and a Q Exactive Focus Orbitrap Mass Spectrometer (MS) was used for analysis. The UHPLC had an ACQUITY BEH phenyl column (2.1 mm × 100 mm, 1.7 μm, Waters, MA, USA) for separation of ATM-carbonyl adducts. The liquid flow rate through the column was set to 0.2 mL/min. The column temperature was stabilized at 30 °C. The autosampler tray temperature was set at 8 °C. 5 μL of the sample was injected into the column. The mobile phase A was 0.1% formic acid in water and mobile phase B was acetonitrile. The instrument was operated in positive electrospray ionization (ESI) mode with a spray voltage of 3.5 kV. Nitrogen was used as sheath, auxiliary, and sweep gas at flow rates of 49, 12, and 2 (arbitrary units), respectively. Chromatographic separation conditions were set via a gradient elution program: 0–1 min, 0%−10% B; 1–3.5 min, 10%−35% B; 3.5–9 min, 35–50% B; 9.0–9.1 min, 50%−0% B; 9.1–11 min, 0% B. The total program runtime was 11 min. Data acquisition and processing were carried out using Thermo Scientific Xcalibur version 4.4. For MS/MS analyses, parallel reaction monitoring (PRM) method was used by MS.

Method for quantitative analysis and validation

In order to obtain calibration curves, limit of detection (LOD), limit of quantification (LOQ), accuracy, intraday and interday precision for the UHPLC-MS method, different concentrations of eight synthesized ATM adducts in water including ATM-butanal, nonanal, acetone, 2-butanone, hydroxyacetaldehye, hydroxy-2-heptanal, pentenal and 4-HHE were

prepared (Figure S1). The coefficients of determination (R^2) were between 0.9918 to 0.9997 as shown in Table S1. LOD and LOQ were calculated from the calibration curves using a linear regression model. LOD = 3.3*(Sy/S) and LOQ = 10*(Sy/S), where Sy is the standard deviation of the response and S is the slope of the calibration curve. Sy is acquired by multiplying the square root of sample numbers with a standard error obtained from regression analysis. The precision results were expressed as relative standard deviations (RSD). RSD values ranged from 0.84% to 7.58% for intraday (n=3) and interday (n=3) tests, while accuracy ranged from 96.1% to 103.9%, indicating good study variation (Table S1).

RESULTS AND DISCUSSION

Characterization of carbonyl compound capture efficiencies by the microreactor

Since low molecular weight carbonyl compounds are ubiquitous in ambient air, deuterated ketones and aldehydes were used to characterize carbonyl capture efficiencies by the microreactors. The deuterated compounds were spiked into a 1L Tedlar bag filled with high purity synthetic air and flowed through the microreactors by applying a vacuum.[29, 30] The capture efficiencies of deuterated saturated ketones (2-butanone-1,1,1,3,3-d5, and 2-pentanone-1,1,1,3,3-d5) and saturated aldehydes (propionaldehyde-2,2-d2, and butyraldehyde-2,2-d2) were determined. Figure S2 shows the relationship between the capture efficiency and the flow rate of spiked air samples flowing from the Tedlar bag through the microreactor. The capture efficiency decreases with an increase in the flow rate. Also, as the molecular weight of the carbonyl compounds increased, the capture efficiency decreased. The flow rate of the breath samples through the chips were fixed at 7 mL/min in order to achieve above 90% capture efficiencies of volatile carbonyl compounds.

Saturated aldehydes, ketones, hydroxy-aldehydes and hydroxy-ketones in exhaled breath

Our previous work has shown that the silicon microreactor technology for breath analysis can be used for detection of lung cancer by observing unique carbonyl VOC biomarkers.[8, 9] However, the FT-ICR MS system used for breath analysis did not have the ability to separate constitutionally isomeric compounds. To enable the separation and identification of isomeric ketones and aldehydes, the UHPLC-MS system was used for analysis of captured carbonyl adducts from breath samples. Exhaled breath samples were collected from twenty subjects using ATM-coated microreactors according to the procedure described in the experimental section. Table 1 lists the study subject information. Fifteen subjects were never-smokers, and the other 5 subjects were current smokers. The effect of smoking on VOCs in exhaled breath is not considered in this study. An important advantage of analysis of ATM-carbonyl adducts from exhaled breath with UHPLC-MS is that there is no need for any further sample preparation and no thermal desorption (as in GC- MS), therefore no concern over thermal degradation. All ATM adducts are positively charged, thus there are no concerns over ionization efficiency. Extracted ion chromatograms were plotted with an isolation window of ±4 ppm. Figure 2 shows the chromatograms of ATM adducts of C5 to C8 saturated ketones and aldehydes in exhaled breath samples in comparison with in- house prepared ATM adducts as standards. The chromatograms showed excellent retention time matches between the breath-derived adducts and the prepared standards. As can be seen, the retention time increases with increasing the number of carbons of the alkyl chain from the same

subgroups of aldehydes and ketones, which is important information for identification of other compounds whose adduct standards may not be easily accessible. *E*, *Z* isomers were found for the ATM-carbonyl adducts that had more than 6 carbons. Ketones generally eluted before the corresponding isomeric aldehydes.

Using the ESI positive mode, we were able to detect saturated subgroups of aldehydes (C1 to C12) and ketones (C3 to C8) ($C_xH_{2x}O$), hydroxy-aldehydes (C2 to C10) and hydroxy-ketones (C3 to C16) ($C_xH_{2x}O_2$), unsaturated subgroups of 2-alkenals (C3 to C10) ($C_xH_{2x-2}O$), and hydroxy-2-alkenals (C3 to C9) ($C_xH_{2x-2}O_2$). Table 2 shows the retention times, concentration ranges, median and mean of four subgroups of saturated carbonyl compounds detected in exhaled breath. It should be noted that the concentrations listed in Table 2 and 3 represent nanomoles of carbonyl compounds in one-liter gaseous breath samples. However, the calibration curves in Fig. S1 and the LOD and LOQ in Table S1 reflect aqueous preparations of purified ATM adduct standards, the latter of which would be free from any potential matrix effect. Volatile carbonyl compounds are often labile, with limited commercial availability and short shelf lives. Therefore, ATM-acetone-d6 was used as IR for quantitation of the detected carbonyl compounds. The quantification was done by multiplying ATM-adduct peak area to the IR peak rata ratio with the amount of added IR. This method is imperfect and can introduce error. Although the absolute intensity of the MS signal of ATM adducts results primarily from the presence of a cationic ammonium headgroup common to all ATM adducts, the compositional differences among carbonyl analytes will introduce some variation in the theoretical curves of MS signal versus concentration. A more accurate quantification should be obtained from calibration curves. We are gratified to see that with just one-liter of exhaled breath collected for each subject, the concentrations of recovered ATM-carbonyl adducts were above the LOD and LOQ in Table S1, validating the reliability of our UHPLC-MS method. Acetone had the highest mean and median concentrations of all carbonyl compounds. Breath acetone is affected by changes in dietary macronutrient composition, caloric restriction, exercise, pulmonary factors, smoking and other assorted factors that increase fat metabolism or inhibit acetone metabolism.[37] The concentrations were lower for compounds with longer alkyl chains, which is probably related to their lower volatility and vapor pressure as well as their source abundance decreasing in exhaled breath.[38]

Formaldehyde had the highest level of all detected aldehydes as shown in Table 2. For current cigarette smokers, acetaldehyde levels could be even higher than formaldehyde in exhaled breath because of higher levels of acetaldehyde in cigarette smoke.[10] Furthermore, nonanal had the second highest median concentration followed by acetaldehyde, propanal and hexanal. Nonanal and hexanal are produced from peroxidation of unsaturated ω3, ω6 fatty acids.[6, 32] Nonanal has been reported as the most abundant aldehyde among the C4 to C10 aldehydes in human breath.[39]

A review of published data on detected aldehydes indicates that in general the detected aldehyde concentrations in exhaled breath were less than 1 ppb.[39] The data in Table 2, however, indicates that formaldehyde, acetaldehyde, propanal, pentanal, hexanal, and nonanal have mean concentrations higher than 1 ppb. The discrepancy may be attributable to the limitations of the methodology used in previous reports. The literature reveals that

solid phase microextraction (SPME) with either physical adsorption or chemical reaction was used most often for extraction of aldehydes in exhaled breath and GC-MS was then used for analysis of these compounds.[10,11,39,40] Furthermore, the literature on detected saturated ketones in breath, from acetone to nonanone, shows that only 2-butanone, 2-pentanone, and heptanone were above detection limits when using GC coupled to a triple quadrupole mass spectrometer for analysis.[40] In contrast, the present method enabled the determination of ketones from acetone to octanone. Many published works indicate that diabetic subjects have much higher acetone levels than healthy controls,[1–6] while lung cancer patients have higher levels of 2-butanone and 2-pentanone in their exhaled breath.[29, 30, 41] Therefore, quantitative methods for analysis of these saturated ketones and aldehydes in exhaled breath can be expected to facilitate noninvasive detection of the corresponding diseases.

Saturated hydroxy-aldehydes from hydroxy-acetaldehyde (C2) to hydroxy-decanal (C10) and hydroxy-ketones from hydroxy-acetone (C3) to hydroxy-hexadecanone (C16) were detected and their concentration ranges were determined. Hydroxy-acetaldehyde had the highest level of all hydroxy-aldehydes, close to acetaldehyde in exhaled breath. Most of the hydroxy-aldehydes and hydroxy-ketones have concentrations below 1 ppb (or 0.041 nmol/L) except hydroxy-acetaldehyde, hydroxy-acetone and 3-hydroxy-2-butanone. The hydroxy-aldehydes were predicted as arising from peroxidation of unsaturated fatty acids, but no hydroxy-ketone subgroup was similarly predicated from the mechanistic study.[32] To date, only hydroxy-acetaldehyde and 3-hydroxy-2-butanone have been reported in exhaled breath.[8, 9, 12, 40–42] These two compounds were reported to have higher levels in the exhaled breath of lung cancer patients compared to that in healthy controls.[8, 9, 12, 41, 42]

Unsaturated aldehydes and hydroxy-aldehydes

Table 3 shows the retention times, concentration ranges, median and mean of 2-alkenal, and unsaturated hydroxy-alkenals detected in exhaled breath. 2-Alkenals from acrolein (C3) to decenal (C10) were detected. Unsaturated hydroxy-aldehydes from hydroxy-propenal or MDA (C3) to 4-hydroxy-2-nonenal (4-HNE) (C9) were also detected. 4-HHE had the highest level among the hydroxy-alkenal subgroup. In general, all 2-alkenals and hydroxy-alkenals have concentrations lower than 1 ppb except 2-pentenal and 4-HHE. 2-Alkenals from C9 to C14 and 4-hydroxy-2-alkenals from C6 to C16 have been reported in exhaled breath condensate samples.[31] Both 2-alkenal and 4-hydroxy-2-alkenal subgroups were predicted in exhaled breath from peroxidation of unsaturated fatty acids.[40] Acrolein was reported in exhaled breath condensate samples.[43, 44] Elevated acrolein and MDA in exhaled breath are important indicators of oxidative stress and inflammation.[14–16] Elevated MDA, 4-HHE and 4-HNE in exhaled breath condensates were reported as biomarkers of COPD and lung cancer.[43, 44] Reviews of aldehydes in exhaled breath clearly indicate that these compounds are closely related to diseases.[6, 7] Thus, quantitative analysis of unsaturated aldehydes in exhaled breath will be critical for developing a diagnostic tool for detection of these diseases.

Structure identification of detected carbonyl subgroup compounds

Rasche et al. introduced fragmentation trees as a method of annotating tandem mass spectra to identify unknown small molecules.[45] In the present work, tandem mass spectra were

obtained to confirm the identity of the compounds based on both retention times and fragment spectra and were used for generating fragmentation trees for the compounds analyzed.

Heptanal is a possible lipid peroxidation product of both ω−6 and ω −7 fatty acids.[6] Several groups have reported heptanal as a biomarker of lung cancer in exhaled breath.[10, 11] Figure 3 shows the fragmentation pathway for the m/z feature identified as the ATM adduct of heptanal. To determine which peak registering an m/z of 215.2114 is the ATM adduct of heptanal as opposed to the ATM adduct of 2-heptanone, tandem MS spectra were acquired from breath samples for the m/z 215.2114 peaks and compared with the MS/MS spectra obtained from the corresponding standards. The collision energy of 20 eV was used to perform the tandem MS experiments. Figures 4(a) and 4(b) were obtained from prepared standards of ATM-heptanal and ATM-2-heptanone, respectively. Figures 4(c) and 4(d) were obtained from an exhaled breath sample at m/z of 215.2114 tentatively identified as heptanal and 2-heptanone. ATM-heptanal and ATM-2-heptanone showed very similar fragmentation ions except that fragment ion m/z 104.1070 only arises from the ATM-heptanal adduct. Activation of oxime ethers derived from aldehydes often results in nitrile formation through loss of the aldoxime hydrogen. This elimination reaction to form a nitrile cannot occur with oxime ethers derived from ketones (Scheme S1).[46, 47] The same fragment m/z of 104.1070 is also seen for ATM-pentanal, but not for ATM-2-pentanone, which also cannot eliminate in this manner (Fig. S3). Figure S4 shows good matches of retention times and MS^2 analysis of ATM-hydroxyacetaldehyde standard and ATM-hydroxyacetaldehyde in exhaled breath samples. Both 2-hydroxy-butanal and 3-hydroxy-2-butanone were detected in exhaled breath samples based on comparison with the retention times of their standards as shown Fig. S5. Further structural confirmation of these two compounds was done by MS^2 analysis of ATM adducts of 2-hydroxy-butanal and 3-hydroxy-2-butanone standards and 3-hydroxy-2-butanone in breath samples as shown Fig. S6. The signal of ATM-2-hydroxybutanal in breath sample is too low to perform MS^2. The fragment ion at m/z 104.1071 was also observed in both ATM-hydroxyaldehyde (Fig. S4) adducts and hydroxyketone adducts (Fig. S6). The presence of an alpha-hydroxy group in these cases can be expected to facilitate formation of this fragment via oxygen-assisted elimination of acetonitrile (Scheme S2).[48] The retention times and MS^2 analysis of ATM adducts of pentenal (Fig. S7) and 4-HHE (Fig. S8) likewise show good matches between the standards and breath samples. Most of the detected alkenals and hydroxy-alkenals in exhaled breath samples were confirmed by using their standards with retention times in Table 3 and MS^2 analyses. These unsaturated aldehydes were produced from peroxidation of unsaturated fatty acids.[6,7,32]

CONCLUSIONS

A MEMS-fabricated silicon microreactor was used to derivatize carbonyl compounds in exhaled breath and UHPLC-MS was used to analyze and identify the adducts. A very broad range of carbonyl compounds including saturated aldehydes, ketones, hydroxy-aldehydes, hydroxy-ketones, and unsaturated aldehydes, and hydroxy-aldehydes were detected. The concentration ranges for these carbonyl sub-types were determined. ATM-derivatized ketone and aldehyde isomers from exhaled breath were separated by UHPLC. Retention times and MS^2 spectra of compounds detected in breath samples were compared with standards for

compound identification. The MS^2 spectra and fragmentation trees were shown to provide valuable complementary information. This approach of combining microreactor technology for capture of carbonyl compounds from exhaled breath and UHPLC-MS for adduct analyses could be a powerful method for quantitative analysis of carbonyl compounds in exhaled breath. The more accurate quantifications might improve breath analysis for detection of lung cancer, COPD, or infectious respiratory diseases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENT

Research reported in this publication was supported by the National Cancer Institute of the National Institutes of Health under Award Number R21CA229057 and the National Center for Advancing Translational Sciences of the National Institutes of Health under Award Number U18TR003787. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. The authors gratefully acknowledge Dr. Bradley Hart at Thermo-Fisher Scientific for the generous support of using the Thermo-Fisher UHPLC-MS system for developing the analytical method.

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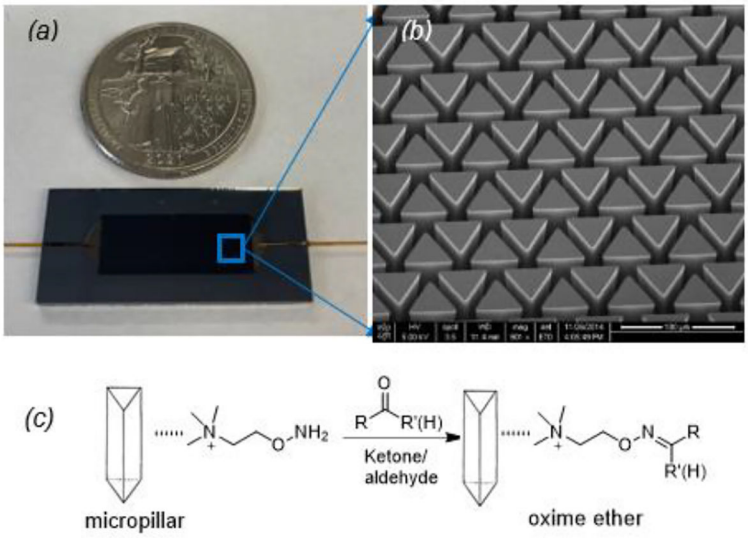

Figure 1.

(a) Optical micrograph of a fabricated microreactor compared with a dime coin; (b) an SEM picture of the micropillar array; and (c) a schematic of ATM coated on micropillars and reaction with ketones and aldehydes to form corresponding oxime ether adducts.

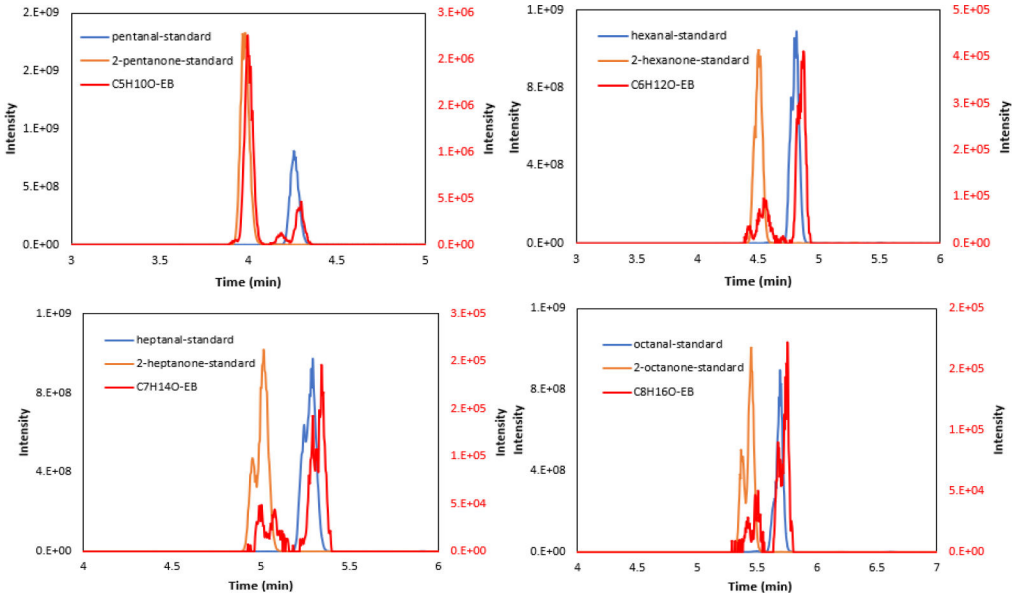

Figure 2.

Extracted chromatograms for saturated ketones and aldehydes obtained from ATM-adduct standards of C5 to C8 carbonyls (blue and orange, y-axis on the left) and exhaled breath (EB) samples (red, y-axis on the right) by UHPLC-MS.

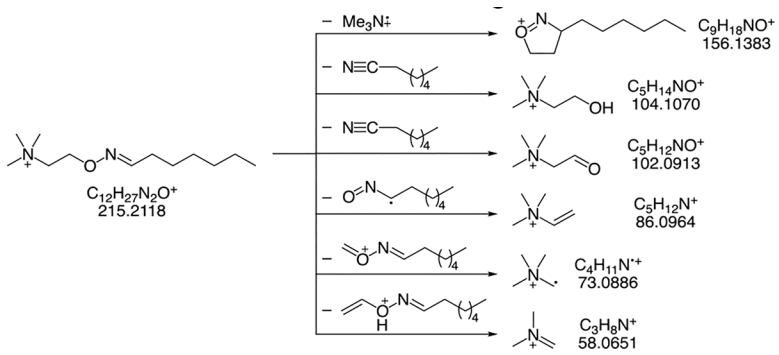

Figure 3.

Fragmentation pathways obtained from the MS/MS spectrum of the m/z feature 215.2114, identified as the ATM adduct of heptanal. Exact masses depicted are theoretical values and are within ±0.0015 amu of the observed value for each fragment ion.

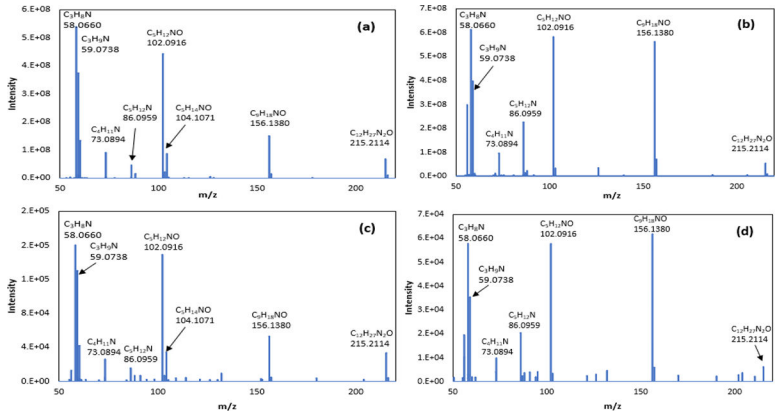

Figure 4.

MS/MS spectra obtained from standards (a, b) and from exhaled breath (c, d) for the compounds identified as ATM-heptanal (RT = 5.33 min, Figure 2) and ATM-2-heptanone (RT = 5.02 min, Figure 2), where RT = retention time.

Table 1.

Study subject information

	All subjects (N=20)	Male (N=10)	Female (N=10)
Age (years)	26–75	34–59	26–75
White race	16	8	8
Height (cm)	150–198	173–198	150–173
Weight (kg)	58–118	68–118	58–102
Current smoker	5	1	4

Table 2.

Concentrations of saturated carbonyl compounds detected in gaseous exhaled breath samples by UHPLC-MS[a]

Saturated aldehydes ($C_xH_{2x}O$)						
Carbon atoms	**m/z**	**acc. (ppm)**	**RT (min)**	**Conc. range (nmol/L)**	**Conc. median (nmol/L)**	**Conc. mean (nmol/L)**
1	131.1177	1.144	1.38	0.544–10.054	4.740	4.484
2	145.1333	1.516	2.06	0.104–1.998	0.358	0.482
3	159.1489	1.634	3.10	0.065–0.313	0.170	0.165
4	173.1645	1.848	3.64	0.113–0.323	0.206	0.203
5	**187.1802**	1.710	4.23	0.050–0.564	0.086	0.116
6	**201.1957**	2.038	4.82	0.050–0.307	0.145	0.153
7	**215.2114**	1.998	5.28/5.33	0.019–0.149	0.054	0.059
8	**229.2269**	2.356	5.68/5.76	0.024–0.187	0.075	0.076
9	243.2426	1.932	6.12/6.21	0.365–2.245	0.871	0.919
10	257.2582	2.216	6.96/7.17	0.014–0.371	0.061	0.098
11	271.2740	1.585	8.25/8.45	0–0.092	0.005	0.013
12	285.2893	2.524	9.15/9.30	0–0.081	0.002	0.008
Saturated ketones ($C_xH_{2x}O$)						
Carbon atoms	**m/z**	**acc. (ppm)**	**RT (min)**	**Conc. range (nmol/L)**	**Conc. median (nmol/L)**	**Conc. mean (nmol/L)**
3	159.1489	1.634	2.99	1.512–27.324	11.638	11.5
4	173.1645	1.848	3.45	0.414–3.447	1.868	1.955
5	**187.1802**	1.710	3.94	0.076–1.403	0.886	0.845
6	**201.1957**	2.038	4.47/4.50	0.001–0.043	0.023	0.024
7	**215.2114**	1.998	4.95/5.02	0.003–0.034	0.012	0.015
8	**229.2269**	2.356	5.37/5.45	0.002–0.016	0.009	0.009
Saturated hydroxy-aldehydes ($C_xH_{2x}O_2$)						
Carbon atoms	**m/z**	**Acc. (ppm)**	**RT (min)**	**Conc. range (nmol/L)**	**Conc. median (nmol/L)**	**Conc. mean (nmol/L)**
2	**161.1281**	1.986	1.29/1.36	0.143–0.619	0.370	0.364
3	175.1438	1.770	1.83/2.14	0.006–0.067	0.025	0.031
4	**189.1594**	2.009	3.07/3.13	0.004–0.045	0.018	0.019

5	203.1751	1.624	3.32/3.48	0.001–0.005	0.004	0.004
6	217.1906	2.302	3.56/3.84	0.002–0.011	0.007	0.007
7	**231.2061**	2.465	4.37/4.49	0.027–0.093	0.033	0.038
8	245.2218	2.365	5.00	0–0.001	0	0
9	259.2374	2.314	5.39	0–0.003	0.001	0.001
10	273.2529	2.818	5.75	0–0.006	0.001	0.001

Saturated hydroxy-ketones ($C_xH_{2x}O_2$)

Carbon atoms	m/z	acc. (ppm)	RT (min)	Conc. range (nmol/L)	Conc. median (nmol/L)	Conc. mean (nmol/L)
3	175.1438	1.770	1.42	0.022–0.070	0.047	0.048
4	**189.1594**	2.009	2.16/2.33	0.001–0.312	0.035	0.059
5	203.1751	1.624	3.04/3.11	0.005–0.028	0.014	0.014
6	217.1906	2.302	3.19/3.27	0.004–0.026	0.012	0.013
7	231.2061	2.465	3.51/3.79	0–0.021	0.004	0.005
8	245.2218	2.365	4.20/4.41	0.001–0.014	0.006	0.007
9	259.2374	2.314	4.59/4.90	0.001–0.019	0.004	0.005
10	273.2529	2.818	4.95/5.11	0.004–0.109	0.012	0.018
11	287.2686	2.506	5.27	0.001–0.007	0.005	0.005
12	301.2841	2.987	5.56	0.002–0.035	0.021	0.022
13	315.2997	2.918	5.91	0–0.007	0.003	0.033
14	329.3155	2.399	6.55	0–0.007	0.003	0.002
15	-		-	-	-	-
16	357.3465	2.910	10.32	0–0.012	0.002	0.003

Table 3.

Concentrations of unsaturated carbonyl compounds detected in gaseous exhaled breath sample by UHPLC-MS[a]

2-alkenals ($C_xH_{2x-2}O$)						
Carbon atoms	**m/z**	**acc. (ppm)**	**RT (min)**	**Conc. range (nmol/L)**	**Conc. median (nmol/L)**	**Conc. mean (nmol/L)**
3	157.1333	1.527	3.07	0.003–0.032	0.009	0.011
4	171.1489	1.636	3.55/3.63	0.002–0.050	0.011	0.015
5	**185.1646**	1.566	4.04/4.16	0.007–0.048	0.025	0.025
6	**199.1802**	1.607	4.61/4.76	0–0.022	0	0.003
7	213.1956	2.439	5.24	0–0.004	0	0.001
8	**227.2111**	2.905	5.53/5.67	0–0.015	0.002	0.003
9	**241.2269**	2.280	5.96/6.21	0.002–0.041	0.008	0.012
10	255.2424	2.899	7.23/7.47	0–0.001	0	0
hydroxy-2-alkenals ($C_xH_{2x-2}O_2$)						
Carbon atoms	**m/z**	**acc. (ppm)**	**RT (min)**	**Conc. range (nmol/L)**	**Conc. median (nmol/L)**	**Conc. mean (nmol/L)**
3	173.1282	1.532	3.01	0.003–0.026	0.008	0.009
4	187.1438	1.502	3.11	0.001–0.011	0.005	0.006
5	201.1594	1.496	3.30	0.001–0.018	0.002	0.003
6	**215.1749**	1.988	3.45	0.028–0.095	0.036	0.038
7	229.1911	2.138	3.75	0–0.134	0.002	0.011
8	243.2062	−0.218	4.25	0–0.007	0	0.001
9	**257.2218**	2.220	4.91	0–0.021	0.002	0.003

[a]. MS^2 were obtained for m/z features in bold. acc stands for the mass accuracy calculated as the measurement error of the m/z found to the calculated m/z. RT=retention time. See Supporting Information for detailed chromatography and MS^2 spectra.