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An Evaluation of Fourier Transform Infrared (FTIR) Spectroscopy for Detecting Organic Solvents in Expired Breath

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The aim of this study was to test the performance of gas-phase FTIR analysis on human breath samples. Ten volatile organic compounds (VOC) were examined for applicability to FTIR spectroscopy (ethanol, ethylbenzene, n-hexane, methyl ethyl ketone, methyl tert-butyl ether, m-xylene, 1,1,1-trichloroethane, trichloroethylene, tetrachloroethylene, and toluene). Three sets of detection limits (LOD) were determined for comparison. LOD_1 were generated from partial least squares (PLS) calibration methods using spectroscopic software, LOD_2 from spiked breath samples, and LOD_3 from blank breath samples. Mixed expired breath samples from four subjects were spiked at varying levels with four different VOC (hexane, methyl ethyl ketone, m-xylene and 1,1,1-trichloroethane) to validate spectral data and test overall accuracy. Breath samples spiked with m-xylene also were validated by GC/FID analysis.

PLS-derived LOD_1 ranged from 0.06–2.47 ppm. Spiked breath sample LOD_2 ranged from 0.52–1.21 ppm. Blank breath LOD_3 measurements ranged from 0.17–1.70 ppm, except for ethanol, which had an LOD of 11.2 ppm. Predicted concentrations for carbon dioxide (slope = 1.06), m-xylene (slopes = 1.19, 1.21), and methyl ethyl ketone (slope = 0.93) were fairly accurate, while concentrations were underpredicted for n-hexane (slope = 0.69) and 1,1,1-trichloroethane (slopes = 0.58–0.66).

Keywords Exhaled Breath Sampling, Fourier Transform Infrared (FTIR) Spectroscopy, Volatile Organic Compounds (VOCs), Organic Solvents, Biological Monitoring, Limit of Detection (LOD)

In recent years, biological monitoring has grown into one of the most direct and relevant approaches to exposure assessment. Within the realm of biomonitoring, breath analysis is of interest as a possible estimate of body burden for many volatile organic compounds (VOCs). Breath monitoring, like other biomonitoring methods, can reflect the uptake of contaminants from all routes of exposure, yet is less invasive than blood or urine analysis and supplies a much less complex matrix to analyze.[1] It thus provides distinct comfort and logistic advantages over these other methods, although some workers may express concern over ethanol monitoring without worker consent. When combined with a suitable air monitoring program, one can determine the occupational contribution to internal dose. Presently, the majority of breath sampling methods require a laboratory setting, with the resulting analyses usually requiring several days. The use of a direct reading instrument could greatly enhance the applicability of breath analysis to field use.

The use of a photoionization detector as a direct reading instrument for breath analysis has been evaluated, but the instrument was found to be poorly selective for individual compounds in a mixed atmosphere, with a limit of detection (LOD) for toluene around 5 ppm.[2] However, as early as 1962, infrared methods have been used to detect trichloroethylene in breath, though not in a direct-reading application.[3] Recently, Fourier transform infrared (FTIR) gas phase spectroscopy was used as a semi-direct breath analysis technique for methanol, which shows promise.[4] FTIR spectroscopy provides many advantages for breath monitoring. Because FTIR measures the entire infrared spectrum instantaneously (known as multiplexing), it can provide quick and accurate identification and quantitation of mixture components with multiple compounds at low concentrations, while also quantifying water vapor and carbon dioxide. Furthermore, it has quite good sensitivity with reported LODs near 10–500 ppb, depending on the compound and calibration method.[5,6]

The basic assumption underlying breath analysis is that there is equilibrium of volatile components between the alveolar air and the pulmonary arterial (capillary) blood.[1] The primary goal of all breath sampling is to accurately determine a compound's alveolar concentration, which can then be used as an index of blood level—the most common indicator of body burden. The partial pressure of a contaminant in blood will equal

its partial pressure in the alveolar air when this equilibrium is established, which, for most VOC usually occurs within 0.3 seconds.[7,8] The equilibrium concentrations are related by a compound's blood:air partition coefficient, denoted by K_{BA}. It has been shown that compounds with $K_{BA} > 10$ generally exhibit a good correlation between the venous blood and alveolar air concentrations.[9] In general, compounds with high molecular weights or high boiling points have high K_{BA}, although no simple relationship exists between a compound's physical properties such as molecular weight or boiling point and its K_{BA}.[10]

Many types of breath sampling strategies have been studied,[2,4,7,11–14] and even reviewed.[1] They include end-expired air, alveolar air, mixed expired air, and rebreathed air. Of these, mixed-expired air samples are of particular interest because of their simplicity in acquisition and applicability to FTIR. Mixed expired breath samples have lower amounts of carbon dioxide than other types of breath samples (rebreathed air, breath holding), so there is less interference in the infrared spectrum. Mixed-expired air consists of alveolar air diluted by air retained in the respiratory dead space (mouth, nose, pharynx, trachea, and bronchi).[1] Alveolar air (end-expired air) constitutes about two-thirds of the tidal volume, so the concentration of a solvent in mixed expired air is approximately two-thirds of the concentration in alveolar air (exception: very water-soluble solvents, such as ethanol, where dead space is very small).[1] This is normally true unless dead space air also contains the analyte, so it is of great importance that mixed expired air sampling be done in a clean environment. Assessment of mixed expired air generally employs a carbon dioxide normalizing factor to standardize any variability between breath samples and to adjust for dead space dilution. By obtaining each subject's mean end-tidal carbon dioxide concentration, the mixed expired breath concentration can be converted to its estimated alveolar concentration.[15] Standard carbon dioxide concentrations used as normalization factors range from 5 percent to 5.5 percent.[15–17] Mixed expired breath samples are normalized to each subject's end-tidal breath carbon dioxide concentration by the following formula:

$$[\text{compound}]_{\text{Normalized}} = \frac{[CO_2]_{ET}}{[CO_2]_{\text{Sample}}} \times [\text{compound}]_{\text{Sample}} \quad [1]$$

where $[CO_2]_{ET}$ is the subject's end-tidal breath carbon dioxide concentration, $[CO_2]_{\text{Sample}}$ is the measured carbon dioxide concentration in the breath sample, and $[\text{compound}]_{\text{Sample}}$ is the concentration of the compound measured in the breath sample.[15]

Fourier transform infrared spectroscopy, which first attained commercial use in the late 1960s, is an infrared spectral analysis technique that provides many advantages for measuring complex mixtures, such as breath samples.[18] FTIR instruments differ from traditional dispersive IR instruments in two ways. First, FTIRs have a much higher signal-to-noise ratio because they allow the entire IR beam, rather than a fraction passing through a slit, to strike the sample.[18] Second, FTIR spectrometers can measure all the wavelengths in the IR spectrum at once, in a process called multiplexing that vastly reduces the time needed to acquire a spectrum. Obtaining a spectrum rapidly offers the added benefit of co-averaging many spectra of the same sample. Because most instrument noise is a randomly fluctuating variable in time, co-averaging many spectra reduces noise and increases the distinction of the desired signal. One disadvantage of FTIR instruments, in regards to dispersive IR spectrometers, is that a background spectrum cannot be obtained simultaneously.

A background spectrum must be obtained separately or sequentially from a sample spectrum and then subtracted to produce the desired transmission or absorbance spectrum. Temporal and environmental fluctuations in bench conditions can adversely affect this correction for background. FTIR instruments have already been demonstrated as potential remote sensing equipment for industrial and environmental atmospheres (known as open-path FTIR).[19] Its application to breath monitoring is not as well-developed: the only compound investigated thus far is methanol.[4]

Carbon dioxide and water both have strong infrared absorption features and occur in relatively high concentrations in breath, which may limit considerably the analysis of breath contaminants using FTIR spectroscopy. The regions of a breath spectrum available for quantitation thus are restricted to about 920–820, 1250–1100, 1970–2220, and 3400–2425 cm^{-1}, although significant interference from water overtones begins at 2950 cm^{-1}. This means that only about 30–40 percent of the total mid-IR band has usable information for a typical breath spectrum. Table I shows the VOC included in the study and the regions used for quantitation.

Ten organic solvents were selected for this study using several criteria. Six compounds (ethylbenzene, n-hexane, 1,1,1-trichloroethane (methyl chloroform), tetrachloroethylene (perchloroethylene), toluene, and trichlorethylene represent six of seven compounds found in the 1995–1996 ACGIH® Biological Exposure Indices with exhaled breath analysis as a possible determinant of occupational exposure.[20] The four remaining

TABLE I
VOC selected for study and region of IR spectrum used for quantitation (in wavenumbers)

Compound	Region (cm^{-1})
Carbon dioxide	2081–2074
Ethanol	1150–950
Ethylbenzene	3138–2806
n-Hexane	3013–2808
Methyl ethyl ketone (MEK)	1240–1110
Methyl tert-butyl ethyl (MTBE)	1137–1049
m-Xylene	3100–2813
Tetrachloroethylene (PERC)	940–875
Toluene	3150–2825
1,1,1-Trichloroethane (TCA)	1163–1036
Trichloroethylene (TCE)	865–815
Water	1969–1964

solvents (ethanol, methyl ethyl ketone, methyl tert-butyl ether [MTBE], and m-xylene) were chosen for the purpose of comparing LOD between differing functional groups, because they are common industrial solvents and because ethanol and MTBE can be considered biomonitoring confounders due to the possibility of non-occupational exposure.

To determine whether FTIR shows good promise for use in breath monitoring, measures of its utility were compared with other methods of breath analysis. Two of these measures are a method's limit of detection (LOD) and its accuracy (predictive ability). A basic definition of the LOD is the minimum concentration level that can be determined to be statistically different from an analytical blank.[21] When applied to traditional IR spectroscopy, the LOD is the concentration of analyte that produces a detector response exceeding instrument noise by three times the noise's standard deviation (3 SD_{noise}). At this level, there exists a 7 percent chance of producing a false negative or false positive determination.[22] In this study, FTIR LOD for 10 VOC were estimated in three ways. The first procedure (LOD_1) used the upper 95 percent confidence limit of the intercept from least squares regressions representing predicted versus actual concentrations of reference spectra. The second procedure (LOD_2) used the upper 95 percent confidence limit of the intercept from a least squares regression between actual and FTIR-determined concentrations of spiked breath samples. The third procedure (LOD_3) used the average predicted value from two blank breath samples quantified for each compound. These three procedures of LOD determination have been shown to provide a more likely estimation of the performance characteristics of FTIR than $\bar{x}_{noise} + 3\ SD_{noise}$.[22]

Next, the FTIR's accuracy was assessed by comparing results for spiked samples that were also analyzed independently on a gas chromatograph/flame ionization detector (GC/FID). In this study, four VOC with differing functional groups (hexane, methyl ethyl ketone, m-xylene, and 1,1,1-trichloroethane [TCA]) were used to validate the results from FTIR quantitation methods. All four compound's sample concentrations were determined by injecting known amounts of analyte into known volumes of mixed expired breath. Two sets of mixed expired breath samples (both spiked with m-xylene) were analyzed by GC/FID to verify the sample preparation method.

This work evaluated the premise that FTIR spectroscopy can accurately determine solvent concentrations in mixed-expired breath samples at levels likely to be encountered in industry. The objectives of this study were: (1) to determine the limits of detection for ten volatile organic compounds of differing functional groups using different methods, (2) to validate four of the FTIR quantitation methods with spiked breath samples, and (3) to compare the prediction of mixed expired breath samples spiked with m-xylene between FTIR and GC/FID.

METHODS

The FTIR instrument in this project incorporates a custom-designed gas cell into a standard optical bench. The optical bench is a Nicolet Magna 550 spectrophotometer, which provides 0.5 cm^{-1} wavenumber resolution over the spectral range of 7400 to 550 cm^{-1}. The optical bench is completely sealed and desiccated to insure the integrity of the KBr optics. The bench is fitted with a liquid nitrogen cooled mercury-cadmium-telluride (MCT) infrared detector. The stainless steel gas cell has a 0.48 liter total volume and the optical path is 4.8 meters. The gas cell and associated piping (also stainless steel) are enclosed within Plexiglas and kept at a temperature of 38.5°C (±0.1°) to prevent moisture condensation in the breath sample. The gas cell is also equipped with a two-liter Tedlar bag that serves as a reservoir for the collection of rebreathed breath samples. The inlet and outlet of the cell are fitted with check valves to assure unidirectional sample flow during sample analysis. The purge valve allows the cell to be flushed with dry nitrogen or another suitable gas between breath samples. A schematic diagram of the instrument is shown in Figure 1.

All spectra incorporated a background spectrum taken with the cell filled with dry nitrogen that was collected immediately before the collection of a set of sample spectra. When there were changes in compounds or time intervals longer than fifteen minutes between spectrum collections, a new background was obtained prior to sample spectra collection.

Calibration spectra for carbon dioxide and water were generated using the optical bench. FTIR spectra were acquired by introducing bag atmospheres to the cell using Gilian GilAir5 personal sampling pump connected to the cell outlet. Carbon dioxide atmospheres were created using a compressed gas mixture (10.1% CO_2 in N_2, Scott Specialty Gases) in successively diluted concentrations (using a 2 L Hamilton Super Syringe and dry N_2) in pre-flushed 25 L Tedlar bags. Water spectra were generated by installing a General Eastern Hygro M1 dew point hygrometer onto the downstream port of the gas cell. A 25 L Tedlar bag of N_2 was kept in a styrofoam box furnished with a heating pad to keep bag temperatures above 37°C.

Successive microliter amounts of distilled water were injected into the bag. At each injection stage, the atmosphere was allowed to equilibrate, then drawn into the cell where it was analyzed. It was then drawn through the hygrometer to determine the dew point. The dew point temperature data were converted to volume fraction water vapor concentrations using a psychometric table.

All reference VOC spectra were obtained from searching available spectral libraries. Spectra for ethylbenzene, hexane, methylene chloride, m-xylene, methyl ethyl ketone, tetrachloroethylene, trichloroethylene, and toluene were obtained by downloading FTIR spectra from the Environmental Protection Agency/Arnold Engineering Development Center (EPA/AEDC) Spectral Database, located at the Web site (http://www.epa.gov/ttn/emc/ftir/welcome.html). All reference spectra selected from this site were collected at 25°C and deresolved to 0.5 cm^{-1} resolution. Spectra for ethanol were obtained from EXAMS (Expert Air Monitoring System, James B. D'Arcy, Automotive Safety and Health Research, General Motors Corporation).

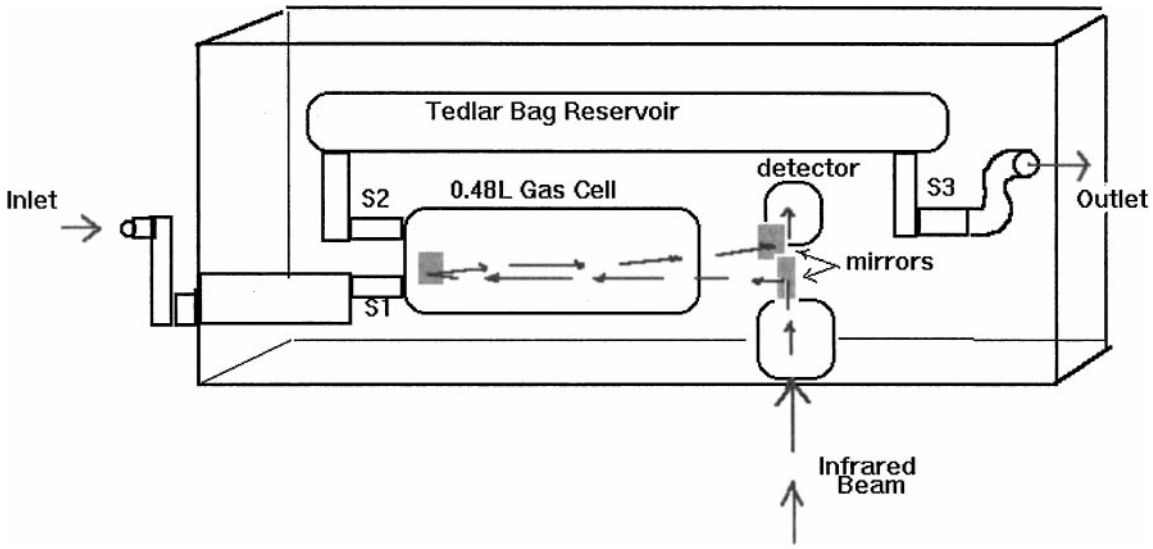

FIGURE 1

Side-view diagram of the gas cell assembly. All piping is stainless steel, except outlet, which is Tygon®. S1, S2, and S3 are solenoid valves to provide unidirectional sample flow and control between mixed expired and re-breathing sample modes.

Spectra were analyzed using a spectral software program (Galactic Industries PLSplus/IQ™ for GRAMS). A separate quantitation method was constructed for each compound. Each method was designed to measure carbon dioxide, water vapor, and the VOC of interest, and used a total of 14–20 calibration spectra. Five calibration spectra of carbon dioxide ranged from 0 to 10.1 percent v/v. Five calibration spectra for water vapor ranged from 2.1 to 4.2 percent v/v. Reference spectra for the selected VOC ranged from 4–10 in number and several orders of magnitude in concentration range to bracket expected breath concentrations (below expected LOD levels and above levels associated with "likely" occupational exposure).

A separate quantitation method was constructed solely for carbon dioxide, because much better accuracy could be achieved alone than when included into a VOC's method. Before being incorporated into a quantitation file, all spectra were first converted into file types that could be handled by the software (using the GRAMS file converter). They then had to be matched for resolution and data range. Ethanol spectra were quantified at 1 cm^{-1} resolution and a data range of 3400–550 cm^{-1}. All other spectra were quantified at 0.5 cm^{-1} resolution and a data range of 4000–550 cm^{-1}.

Spectra that represented differing concentration levels in our instrument were required for each VOC's quantitation method. Independent primary spectra for each VOC were available from libraries for at least two different concentrations (all at 25°C) but at several different path lengths. A set of standard spectra representing concentrations in our gas cell (4.8 m) over the instrument range for occupational exposure were obtained by multiplying a compound's primary spectrum by differing constant factor levels. This multiplication adjustment assumes that the absorbance changes linearly in proportion to the concentration-path length product.

The FTIR quantitation method used in this study is a type of factor analysis known as Partial Least Squares (PLS). PLS is a robust FTIR quantitation method that is relatively unaffected by the presence of impurities and can identify multiple components in a mixture.[18] It is the method of choice for spectra that contain mixtures with overlapping peaks and interactions between sample components. In addition, it is ideally suited for noisy spectra (like those of breath) and spectra that may contain artifacts. PLS uses scores and factors that represent variation in the calibration spectra, not the spectra themselves.[18] These variation spectra are then used to construct the calibration curve. PLS quantitation method factors were optimized by running maximum factor cross validation models to obtain the optimum number of factors, then reconstructed using these optimized factors. The cross validation model was a diagnostic algorithm that repeatedly recalculates the calibration curve leaving one standard out at a time.

Human subjects provided blank breath samples for the purpose of spiking with VOC. Four (3 male and 1 female) human subjects were recruited under a protocol approved by the University of Washington Human Subjects Research Committee. These subjects were non-smoking adults between the ages of 23 and 28 and provided informed consent prior to participation in this experiment.

Prior to sample collection, all Tedlar bags were flushed with near maximum volumes of dry nitrogen, placed in an incubator at 41°C for a minimum of 45 minutes, and then emptied via the laboratory vacuum. This flushing procedure was repeated four times. During sample collection, bag volumes were

determined by displacement of air from an airtight Plexiglas box using a 13.5 liter respirometer (Warner E. Collins, Inc.). Two of the subjects were asked to exhale normally into nine 10-liter Tedlar bags. The two other subjects were asked to exhale normally into four 10-liter Tedlar bags and five 25-liter Tedlar bags. After collection, all bags were homogenized by vigorous agitation. Carbon dioxide concentrations were then measured using an Ametek CD-3A direct reading carbon dioxide meter, removing approximately 0.03–0.06 L of the sample volume during this measurement. The bags were then placed in the incubator for one hour to ensure complete volatization of the injected VOC and good mixing of the VOC and the breath atmosphere.

After incubation, the bags were spiked (injected) with appropriate amounts of liquid contaminant to achieve the desired final breath concentration. To increase the accuracy and precision of the amount injected, all contaminants were dissolved in larger liquid volumes of carbon disulfide (CS_2). It was determined that this solvent does not interfere with the infrared absorbances of the compounds of concern.

Separately, mean end-tidal carbon dioxide levels for each subject were obtained by having the subject sit in a comfortable upright position and relax while breathing through a mouthpiece and wearing a nose clip. Exhaled air was continuously monitored using an Ametek CD-3A direct reading carbon dioxide analyzer, which was connected to the midstream sampling port on the mouthpiece. Data were recorded electronically utilizing Labview 2.2 software for the Macintosh. Mean end-tidal carbon dioxide concentrations were calculated by averaging the concentration maxima of approximately 60–90 consecutive breaths.

Validation of spiked breath concentrations by GC/FID were performed only for m-xylene. The m-xylene validation served as a relative confirmation of the spiked breath concentrations for the other three compounds. After one hour of incubation following spiking, the bags' contents were drawn through SKC 100/50 mg charcoal tubes using Gilian pumps while still in the incubator. Pump flow rates were calibrated using a bubble buret and a stopwatch and ranged from 0.46 to 0.49 L/min. Sample volumes were corrected to body temperature.

Charcoal tubes were subsequently desorbed with a carbon disulfide (Omnisolve spectrophotometry grade, EM Science) solution containing 50 ppm n-propylbenzene (Aldrich) as an internal standard. The front and back sections of charcoal from each sample and bag blank tube were transferred to 4 mL glass vials with Teflon-lined caps. The glass vials were pre-filled with desorption solution using a Brinkman Dispensette dispenser set to deliver 2 mL. Desorbates were sonicated for 5 minutes. After 1 hour at room temperature, desorbates were transferred to 1.5 mL autosampler vials and analyzed for m-xylene on a Hewlett-Packard 5890 Series II Gas Chromatograph with a Flame Ionization Detector (GC/FID), equipped with J&W Scientific DB5MS wax column (30 m length, 0.25 μm film thickness, 0.25 mm inside diameter). Species concentration in simulated mixed exhaled air was then normalized to simulated alveolar concentration utilizing each subject's mean end-tidal CO_2 normalizing factor.

The hexane, MEK, m-xylene, and TCA levels in the spiked breath samples were chosen to represent likely exhaled breath concentrations resulting from corresponding occupational exposures described in the literature. For compounds with corresponding ACGIH BEI's, this value was used for one of the spike samples. For example, TCA in the spiked breath samples (BEI = 40 ppm) was spiked at four levels, 40, 15, 5, and 2 ppm, to span the range from anticipated LOD to likely occupational exhaled breath concentrations.

RESULTS

Prior to comparing the results of FTIR analysis with the concentrations calculated from known amounts of injected analytes, it was necessary to validate the preparation method used to produce simulated breath atmospheres. The concentrations in each bag atmosphere at five levels (0, 0.5, 2, 5, and 10 ppm) for subjects 3 and 4 were validated for m-xylene using GC/FID. There was an excellent correlation between the preparation method calculation and GC/FID results ($r^2 = 0.986$, $y = 0.98x - 0.08$, t-test for intercept = 0, $t = -0.407$, $p > 0.50$, t-test for slope = 1, $t = -0.599$, $p > 0.50$). Subject 3 had one disparate reading, notably at level 4 (preparation method = 4.54 ppm, GC method = 3.23 ppm). There is reason to believe that the preparation method value for this sample is anomalous because the FTIR value for the same sample was close to the GC result (FTIR = 3.67 ppm). Given the excellent agreement for the m-xylene validation experiment, this was taken as acceptable confirmation of the spiked breath concentrations for the other three compounds.

Three sets of LOD results for the selected compounds are shown in Table II. Concentrations in FTIR gas spectroscopy are normally quantified (or reported) in ppm-meters, which standardizes concentrations for spectra collected on different instruments with varying path lengths. The air concentration of a sample is then calculated by dividing the ppm-m value by the path length, which for the cell used here is 4.8 meters. All the LOD values in Table II reflect the actual air concentration after accounting for path length.

The LOD_1 values in Table II were determined by performing least squares regressions on predicted versus actual concentrations of the reference spectra for the calibration files. These LOD_1 values are generated from the cross-validation procedure. To give conservative values, the upper 95 percent confidence limit of the intercept was used as the LOD_1. Student's t-tests were performed and confidence limits were calculated to determine whether the intercepts significantly differed from zero. The LOD_1 for the selected VOC ranged over more than four orders of magnitude and probably are overly optimistic. The extremely low LOD_1 for m-xylene, for example, may be erroneous. In the quantitation method, the training data set (the calibration spectra) used to construct the calibration curve also appears as the validation set. So there is reasonable potential for near-perfect

TABLE II
Three sets of FTIR-determined LOD for 10 VOC

Compound	LOD_1 upper 95% (ppm)	LOD_2 upper 95% (ppm)	LOD_3 Mean (ppm) ± SD
Ethanol	1.34[A]		11.19 ± 2.83
Ethylbenzene	0.38		1.65 ± 1.59
n-Hexane	1.30	0.52	0.17 ± 0.15
Methyl ethyl ketone	2.47	0.91[A]	0.44 ± 0.73
Methyl tert-butyl ether	0.06		−0.34 ± 0.44
m-Xylene	<0.01[A]	1.16[A]	1.69 ± 1.23
Tetrachloroethylene	0.07[A]		0.99 ± 0.29
Toluene	0.48		1.28 ± 1.09
1,1,1-Trichloroethane	1.95	1.21	0.44 ± 0.04
Trichloroethylene	0.48		1.43 ± 0.46

[A] $p < 0.01$ for intercept = 0.

agreement between the prediction method and the validation spectra (which *are* a subset of the training spectra).

Another data set using spectra from independent spiked breath samples were collected to determine LOD values to assess each method's accuracy. Table II depicts the LOD_2 calculated for each of the four compounds used for validation by the spike injection method. The LOD_2 values were obtained by performing a least squares regression at four concentration levels between the FTIR-determined concentrations and those calculated from the spiking method. The LOD_2 ranged from 0.52 ppm for hexane to 1.21 ppm for m-xylene. As shown, the LOD_2 results are in much closer agreement with each other than the LOD_1 values.

Finally, spectra obtained from blank breath samples were analyzed to create a third set of LOD estimates (LOD_3). These values are the average FTIR-predicted concentrations of two blank mixed expired breath samples from each of the four subjects. This analysis gives perhaps the best measure of the FTIR instrument's minimum detection ability, because it is synonymous with the general definition of the LOD—the measurement of an analytical blank.

As can be seen from Table II, ethanol has a high LOD_3 (11.19 ppm) compared to the other nine VOC. This is not consistent with the previous two LOD procedures, where all ten VOC LOD were in closer agreement. There was also no general pattern within functional groups, as the chlorinated compounds LOD_3 ranged from 0.4–1.4 ppm, although the aromatic compounds were in good agreement (1.65 ppm, 1.69 ppm, and 1.28 ppm, for ethylbenzene, m-xylene, and toluene, respectively). The small negative LOD_3 associated with MTBE (−0.34 ppm) probably is attributable to the FTIR registering small negative absorbances in the region used for quantitation in the blank breath samples. Negative LOD values can arise from random noise in the sample spectrum, errors in the background collection or from the calibration method regression line not passing through the origin.

A separate quantitation method was constructed for carbon dioxide because including it in with the VOC's quantitation resulted in poor accuracy. This allowed a comparison between FTIR-determined CO_2 concentrations and those by the Ametek CO_2 Analyzer for all breath samples. A very close correlation between FTIR and the Ametek CO_2 values is depicted in Figure 2. The mean end-tidal CO_2 measurements for subjects 1–4 were 5.28 percent, 4.67 percent, 4.75 percent, and 4.55 percent, respectively. In a study where mixed expired breath samples were obtained from truly exposed individuals, the mean end-tidal CO_2 values would have been used to adjust mixed expired breath levels to alveolar levels. When these values are applied to this study's data, they suggest that the FTIR could detect a sightly lower level of body burden if the same analyses were performed directly on alveolar air.

Table III illustrates the slopes and intercepts of regression analyses between injection method values and those determined by the FTIR quantitation methods, across all four validation compounds. As shown, quantitation methods for m-xylene and MEK had reasonable accuracy (slope ~ 1) over their concentration ranges. However, the methods for hexane and TCA consistently underpredicted the spiked breath concentration levels. Overall, the FTIR methods tended to underestimate the higher concentration levels across all four compounds. This underprediction most likely arises from non-linearity in the IR absorbance features that was not adequately modeled in the reference spectra standards.

Figure 3 depicts the variation in measured differences over increasing concentration levels for all subjects. As shown, the largest underestimate of TCA is at the highest concentration level, and this is also the case for hexane. The m-xylene quantitation method consistently overestimated concentrations at all levels, and slightly more so at the highest level. The highest concentration level for all compounds was invariably the most underestimated for all subjects.

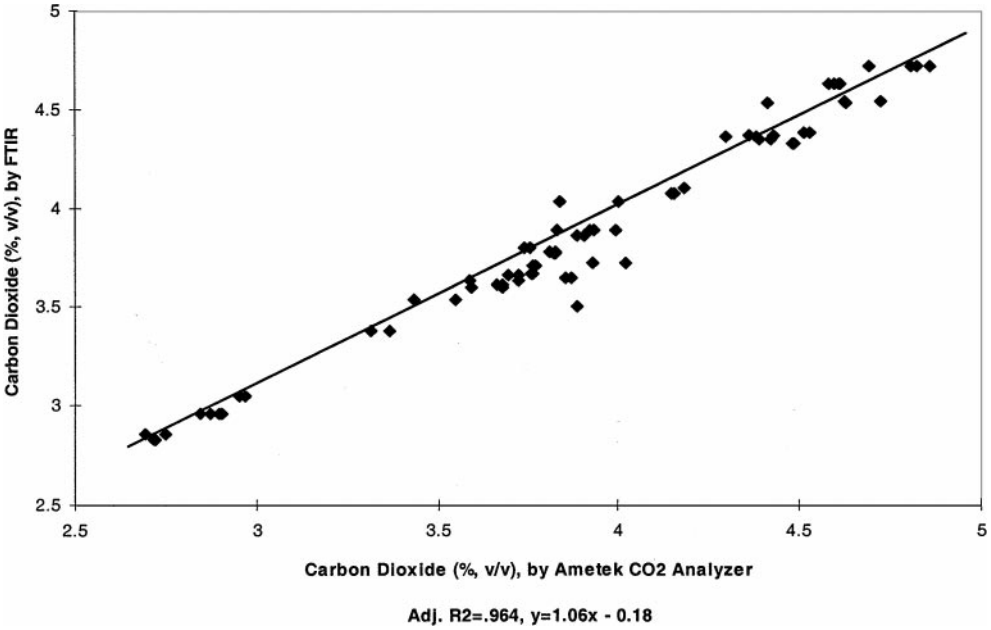

FIGURE 2
Scatter plot of FTIR vs. Ametek carbon dioxide measurements.

Analyses of variance (ANOVA) of the measured difference between FTIR-determined and injection methods were run for three factors: compound, concentration level, and subject. These revealed that when the measured differences between the FTIR and injection methods were grouped by compound and concentration level, there was significant variation within each group (p-values < 0.01 each). Additionally, it revealed a two-way interaction between compound and concentration level that accounts for a large percentage (36%) of the total variation in the data (p-value < 0.01). Thus, there was no consistency in the variation of measured differences between compounds as the concentration level changed. In contrast, the second ANOVA of FTIR and injection method differences run by subject revealed that, as hoped, there is little between-subject variability in these measured differences, and as such, the subject factor contributes little to the total variation in the data (p-value $= 0.424$).

To further assess the accuracy of the FTIR quantitation methods, the results for mixed expired breath samples containing m-xylene were compared to results obtained by charcoal tube desorption with GC/FID. Both analyses were conducted on the same set of breath samples. The m-xylene method had reasonable accuracy for subjects 3 and 4 (slope 1.09 adjusted $r^2 = 0.960$, slope 1.13 adjusted $r^2 = 0.957$, respectively) but still tended to modestly overestimate values.

DISCUSSION

This study determined three LOD values for breath samples using FTIR for a variety of volatile organic solvents that span

TABLE III
Intercepts and slopes from regression lines between FTIR and injection method concentrations

Compound	Subject	Intercept (ppm)	P-value	Slope (unitless)	P-value[A]
n-Hexane	1	0.02	0.94	0.69	<0.005
Methyl ethyl ketone	2	0.55	0.01	0.93	<0.10
m-Xylene	3	−1.14	0.01	1.19	<0.05
m-Xylene	4	1.76	0.00	1.21	<0.10
1,1,1-Trichloroethane	1	0.97	0.00	0.58	<0.001
1,1,1-Trichloroethane	2	0.57	0.01	0.64	<0.001
1,1,1-Trichloroethane	3	0.47	0.05	0.62	<0.001
1,1,1-Trichloroethane	4	0.53	0.19	0.66	<0.001

[A]P-value for test of slope, using $t = \frac{x-\mu}{s/\sqrt{n}}$, $\mu = 1$.

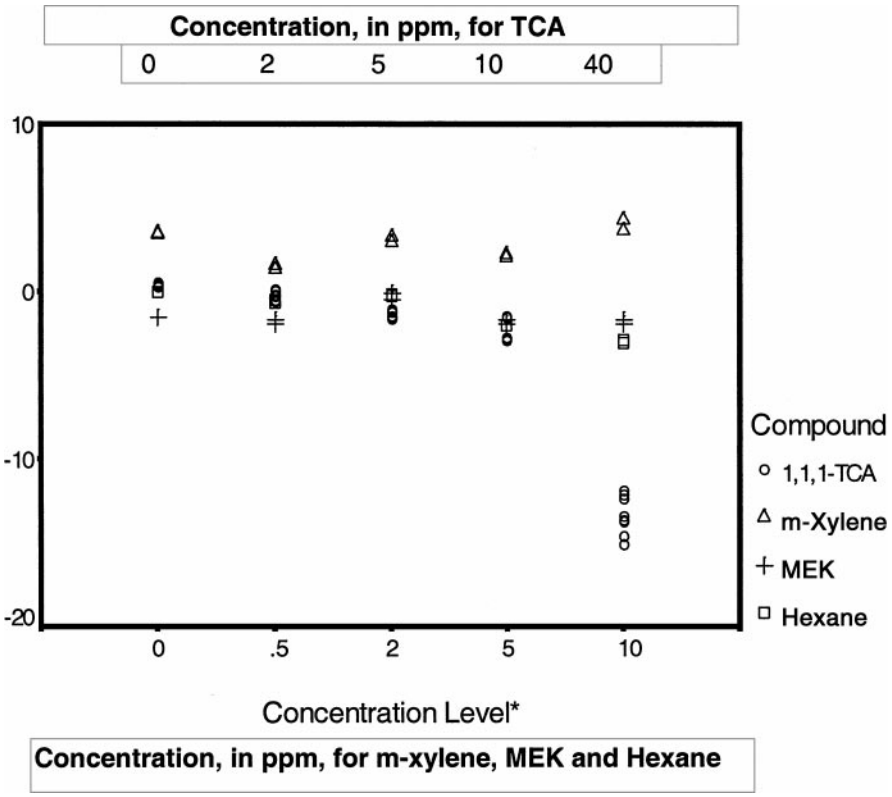

FIGURE 3
Measured difference between FTIR and injection method values versus concentration level, by compound.

different functional groups. The LOD_1 values determined from the quantitation method alone were less reliable and more variable, while the LOD_2 from spiked samples were similar in magnitude to the LOD_3 values determined from blank breath samples. Compared to similar published data, the FTIR-determined LOD_3 for ethanol (~11 ppm) differed by over an order of magnitude from the FTIR-determined LOD for methanol (0.5 ppm) in another study.[4]

Analysis of breath samples appears to present greater challenges and sample variability compared to sampling in ambient air. One study found FTIR-determined LOD *in ambient air* that ranged from 0.007–0.067 ppm for seven of the same compounds used in this study.[5] Clearly, the sensitivity of FTIR to organic solvents is diminished when sampling expired breath in comparison to ambient air. The notable decrease in sensitivity for breath samples probably results in part from the relatively high levels of water vapor, which leads to interference and background variation from water absorbance features and overtones spread over the mid-IR region.

Yet despite these challenges, the LOD values for the FTIR analysis are sufficiently good to allow quantification of all these VOC for many occupational exposures. In the spiked samples, the LOD_2 values were between 10 and 100 times lower than the expected breath concentrations from occupational exposures. In addition, because mixed expired air is a dilution of alveolar air, if true alveolar air were sampled using FTIR, the same LOD would permit detection of a lower level of body burden than if the analysis were performed on mixed expired air.

The FTIR quantitation methods were reasonably accurate at determining CO_2 and MEK concentration levels in breath samples, while modestly overestimating (~20%) m-xylene. In the m-xylene spectra, interference from harmonic overtones due to water vapor probably added to the variability in this quantitation method. Although the quantitation methods used have the ability to determine concentrations for mixtures of analytes, no mixtures of VOC were evaluated. It has been noted that the effects of spectral overlap on LOD and FTIR accuracy are inconsistent.[21] Other researchers have found that analysis of mixtures degraded performance and produced a 2- to 50-fold increase in LOD.[23]

The methods tended to more strongly underestimate hexane and TCA in the spiked samples, typically by about 30–40 percent. Calibration errors for these compounds could have occurred due to nonlinearity with respect to Beer's law for peaks, which typically have high absorbance. The concentration derived from an absorbance spectrum will only be accurate for a linear model at low absorbances. When calibration methods contain standards that span several orders of magnitude, IR spectra have been shown to deviate from Beer's law.[4] The concentration where non-linearity becomes an important factor is not easily predicted and depends on several factors, including the

instrument resolution, the apodization function applied to the spectrum, as well as the natural line width of the absorption features.

Further optimization of the FTIR quantitation methods might improve the overall accuracy of the analysis. Several refinements are possible, such as incorporating reference spectra over a wider concentration range, further optimization of PLS factors, including mixtures in the reference library, and performing a critical review of library reference spectra prior to inclusion. Another refinement that should improve the performance of calibration methods would be to increase homogeneity in sample conditions between reference spectra and sample spectra. Ideally, sample spectra for mixed-expired breath analysis should be compared to mixed-expired breath *calibration standards* that contain differing levels of the compounds in question and are analyzed at the same reference temperature as the breath sample. Rather than using existing libraries for calibration spectra as we have done in this study, it would be preferable to prepare a large set of calibration standards by spiking blank breath samples from various subjects as described here. This should provide a more robust set of calibration standards for breath analysis.

Also, it has been suggested that FTIR spectral quantitation methods should only be constructed using calibration spectra collected on the same instrument.[18] Indeed, the generation of FTIR quantitation methods using spectra from external libraries might seem unjustified. Discrepancies in the spectra can arise from many factors; differences in interferogram processing (phase correction or apodization), spectral file conversion, environmental conditions, detector response, and instrument artifacts all can contribute to errors in the library spectra and quantitation methods.[24] However, IR spectral libraries remain attractive because they offer many advantages in terms of simplicity, standardization, quality control, and cost. Some progress has been made recently to make standardized IR libraries that are useful for quantitative analysis.[25,26]

Prior to the completion of this study, several attempts were made to separate water and carbon dioxide from the breath samples before measurement by FTIR. Columns of Drierite and non-hygroscopic soda lime granules, respectively, were used to remove each of these components. Although substantial fractions of both water and carbon dioxide were removed from breath samples, large amounts of VOC were also removed. Other breath analyses have used cryogenic methods to separate components prior to analysis, but these methods are obviously neither compatible with direct-reading analysis nor applicable to field use and are beyond the scope of this work.

Because it is often not advisable to operate analytical instruments near their LOD, more work remains to be done to determine limits of detection and quantitation for FTIR and organic solvents in breath samples. This work demonstrates that direct reading FTIR methods probably can detect these VOC at levels anticipated for end-of-shift sampling, but may be questionable for overnight or beginning-of-work-week sampling, which typically involves lower concentrations, depending on the toxicokinetics specific to each compound. Elimination of VOC in breath is a function of the partitioning between aqueous and lipid compartments, and of the relative rate of perfusion to each. As a result, a breath sample taken at any time reflects a weighted average of the exposures that occurred during the past 8 to 16 hours (the dominant component) plus exposures that occurred during the past 2 to 4 days (the minor component). Thus, a sample taken at the beginning of the week, after two days without exposure, represents only the minor component from exposures in the last day or two of the previous week, and the breath concentration will be relatively low. This sampling time would be appropriate to estimate cumulative exposures, but would require reduction of the LOD below present levels.

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