DETECTION OF ALDEHYDES IN LUNG CANCER CELL CULTURE BY GAS CHROMATOGRAPHY/MASS SPECTROMETRY AND SOLID-PHASE MICROEXTRACTION WITH ON-FIBER DERIVATIZATION

A Thesis

by

GUANGQING SHAN

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2006

Major Subject: Chemistry

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Approved by:

Chair of Committee,	John W. Bevan
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	Robert Lucchese
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ABSTRACT

Detection of Aldehydes in Lung Cancer Culture by Gas Chromatography/Mass Spectrometry and Solid-phase Microextraction with On-fiber Derivatization. (May 2006)

Guangqing Shan, B.S.; M.S., Shandong University, China

Chair of Advisory Committee: Dr. John W. Bevan

Aldehydes in lung cancer cell culture have been investigated using gas chromatography/mass spectrometry and solid-phase microextraction with on-fiber derivatization. In this study, the poly(dimethylsiloxane/divinylbenzene (PDMS/DVB) fiber was used and *o*-2,3,4,5,6-(pentafluorobenzyl) hydroxylamine hydrochloride (PFBHA) was first loaded on the fiber. Aldehydes in the headspace of lung cancer cell culture were extracted by solid-phase microextraction (SPME) fiber and subsequently derivatized by PFBHA on the fiber. Finally, the aldehyde oximes formed on the fiber were analyzed by gas chromatography/mass spectrometry (GC/MS). Using this method, acetaldehyde decrease was found in both non-small lung cancer cell cultures studied compared to the medium control study. The results of spiking the cell culture with acetaldehyde solution showed that 5 million SK-MES-1 cell lines could consume up to 4.5 uM acetaldehyde in 15-ml medium, and 5 million NCI-H522 cell lines could consume 5.9 uM acetaldehyde in 15-ml medium. The decrease of acetaldehyde may contribute to the metabolism of lung cancer cells. It was proved that GC/MS and SPME

with on-fiber derivatization is a simple, rapid, sensitive and solvent-free method for the detection of aldehydes in lung cancer cell culture.

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With deepest love and appreciation, I would like to thank my family for their encouragement, patience and love.

NOMENCLATURE

GC/MS	Gas Chromatography/ Mass Spectrometry
PFBHA	*o*-2,3,4,5,6-(pentafluorobenzyl) hydroxylamine hydrochloride
SPME	Solid-phase Microextraction
VOC	Volatile Organic Compounds
RSD	Relative Standard Derivation
LOD	Limit of Detection
SIM	Selected Ion Monitoring
SIFT-MS	Selected Ion Flow Tube Mass Spectrometry

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INTRODUCTION

Volatile organic compounds (VOCs), which are mainly blood borne, can provide insights into different biochemical processes in the healthy and the diseased body. Looking at a set of volatile markers may enable recognition and diagnosis of complex diseases such as lung or breast cancer.[1] Breath analysis study showed that far more than 500 different VOCs, principally alkanes and benzene derivatives, had been identified as candidate makers of lung cancer.[2-4] A high level of aldehydes was found both in breath and blood of lung cancer patients. Acrolein was detected in blood from patients with breast cancer. [5] The formaldehyde level from women with breast cancer was higher than that from healthy women. [6] Hexanal and heptanal were also found in both breath [3,4,7] and blood [8-11] of lung cancer patients. It is well understood that the enhanced activity of abnormal cells in vivo results in enhanced levels of certain molecules in blood and breath. [12, 13] Compared to the amount of studies conducted in both breath and blood of lung cancer patients, the information on cancer cell emissions into blood and then into breath is less. Formaldehyde was detected in the headspace above previously lysed breast cancer cells. [14,15] Recently it is discovered that acetaldehyde was released by lung cancer cell lines investigated. [16] Therefore aldehydes are regarded as potential markers of cancer and have been proposed as a diagnosis application.

Gas chromatography/mass spectrometry (GC/MS) was widely applied to the analysis of aldehydes in breath and blood of lung cancer patients.[3, 4, 7-11] However, due to the volatility and trace levels of aldehydes in breath and blood, it is very difficult to detect

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This thesis follows the style of *Rapid Communications in Mass Spectrometry.*

and measure them. *O*-2,3,4,5,6-(Pentafluorobenzyl) hydroxylamine hydrochloride (PFBHA) was first introduced for the derivatization of carbonyls in GC analysis by Cancilla and Hee. [17] The reaction is very fast and the corresponding oximes form in seconds. The aldehydes oximes need to be extracted first and then subjected to analyzed by the GC/MS. Solid-phase microextraction (SPME), introduced by Pawliszyn *et al*., is a new sample technique which can easily transfer the analytes to the GC inlet. [18] This technique is a simple, sensitive and solvent free and it can also concentrates samples. SPME with on-fiber derivatization followed by GC/MS was applied to the analysis of aldehydes in air and water. [19-24] Recently, a serial of papers was published by Deng *et al*. who developed a method using GC/MS and SPME with on-fiber derivatization for analysis of aldehydes in the blood of lung cancer patients. [10, 11, 25]

Lung cancer is a leading cause of cancer-related death because it is difficult to detect in its early stages. It is possible that the increased metabolic rate and the accelerated glycolysis that cancer cells display may result in identifiable molecular makers in exhaled breath. We investigated aldehyde molecular emissions from lung cancer cell culture and hopefully this will bring some benefits for the early detection of lung cancer. The primary goal of this thesis was to detect potential aldehyde marker from lung cancer cells by using GC/MS and SPME with on-fiber derivatization. Aldehydes in the headspace over the cancer cells were extracted by a SPME fiber and reacted with PFBHA on the fiber (see Figure 1). The oximes formed were desorbed and analyzed by GC/MS. Acetone was also studied in the present work because a former student in our

group reported acetone enhancement in the headspace over the lung cancer cell culture by using purge & trap and GC/MS. [26]

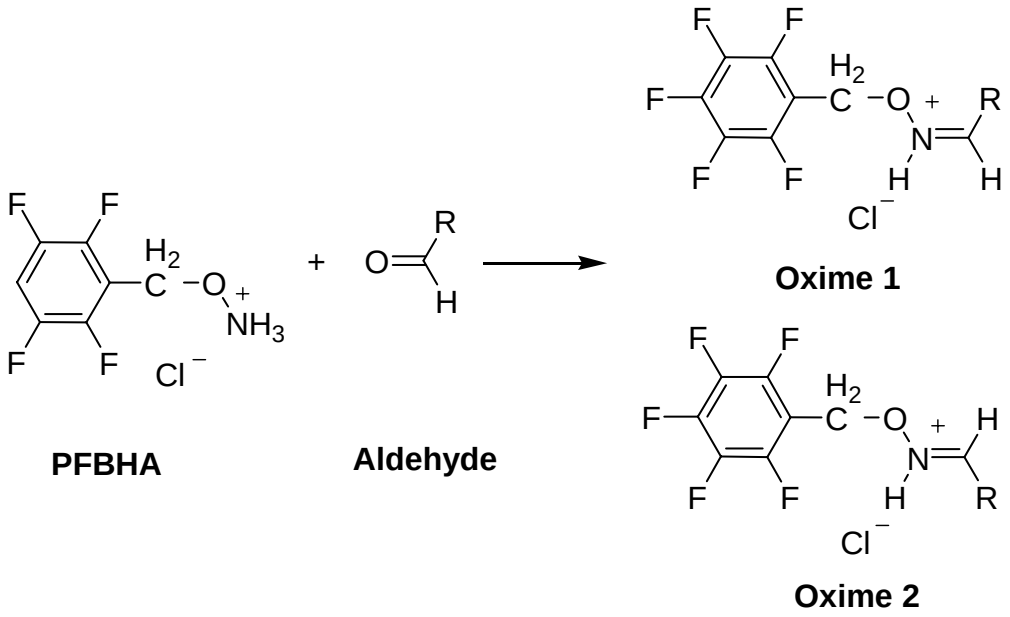

Figure 1. Schematic of the reaction between aldehyde and PFBHA occurring on the SPME fiber.

EXPERIMENTAL

Chemicals and SPME fibers

GC grade organic compounds (purity > 98%) including acetone and acetaldehyde, were purchased from Sigma-Aldrich. *o*-2,3,4,5,6-(pentafluorobenzyl) hydroxylamine hydrochloride (PFBHA) was purchased from Lancaster synthesis Inc. The Solid-phase microextraction(SPME) holder and the 65 um poly(dimethylsiloxane)divinylbenzene(PDMS/DVB) fiber were from Supelco. 40 ml glass vials (Fisher Scientific) with septum is used for both calibration and cell culture. Double distilled water was used for the preparation of acetaldehyde and PFBHA solution.

Calibration solution preparation

Standard stock solution of acetone (10mM) and acetaldehyde (10 mM) were prepared in methanol and stored at 4 ^{o}C for up to 1 week. Because of the easy contamination of acetone from laboratory air and water, very careful procedures were taken for acetone detection. Aqueous solution was avoided because there was a certain amount of acetone even in double distilled water. Acetone calibration was performed in medium because there is no detectable acetone in medium using GC/MS and SPME technique. Standard solutions containing 5 uM, 10 uM, 20 uM and 50 uM of acetone were prepared by spiking 10 mM stock solution to 15 ml medium in a 40 ml vial. Acetaldehyde standard solution containing 0.5 uM, 1uM, 5 uM, 10 uM, and 20 uM acetaldehyde were prepared daily by dilution of 10 mM stock solution with double distilled water. Solution was

allowed to stay at 37 ^{o}C in a incubator for 2 hours to reach equilibrium before it was subjected to analysis.

Gas chromatography/mass spectrometry

All analysis was performed on HP G1800C GCD system. Compounds were separated by using a 30m, 0.25 i.d., 0.25 um film DB-wax fused silica capillary column (Agilent, USA). The carrier gas was ultra pure helium with flow rate of 1.0 ml/min. Splitless mode was used. The injection temperature was set as 250 ^{o}C. The column temperature programs were: initial temperature of 60 ^{o}C, increased to 150 ^{o}C at 6 ^{o}C/min, then increased to the final temperature of 270 ^{o}C at 15 ^{o}C/min. Electron ionization with an electron energy of 70 eV was used. Quantitative analysis was performed by using selected ion monitoring (SIM) with the characteristic ion at *m/z* 181.

Solid-phase microextraction(SPME)

Solid-phase microextraction (SPME) is an extraction technique for organic compounds in aqueous samples, in which analytes are adsorbed directly from the sample onto a fused-silica fiber that is coated with an appropriate stationary phase. While the fiber is inserted in the sample, the analytes partition from the sample matrix into the stationary phase until equilibrium is reached. The fiber is then inserted into the injection port of a gas chromatography where it is heated, and the analytes are rapidly thermally desorbed into a capillary column for analysis. SPME requires no solvents or complicated

apparatus. It can concentrate volatile and nonvolatile compounds, in both liquid and gaseous samples, for analysis by GC, GC/MS, or HPLC. The basic equipment of SPME is simple. As shown in Figure 2, a fused-silica fiber is attached to a stainless steel plunger sheathed by a protective needle. The SPME operation procedure can be described as follows:

Sample extraction

With the fiber retracted, pass the needle through the sample vial septum.

Depress the plunger to expose the fiber to the headspace above the sample.

Analytes adsorb to the fiber in 2 to 30 minutes.

Retract the fiber into the needle and remover the needle from the sample vial.

GC analysis

Insert the needle into the GC injector port.

Depress the plunger, exposing the fiber in the heated zone of the injector to Desorb the analytes onto the column.

Retract the fiber and remove the needle.

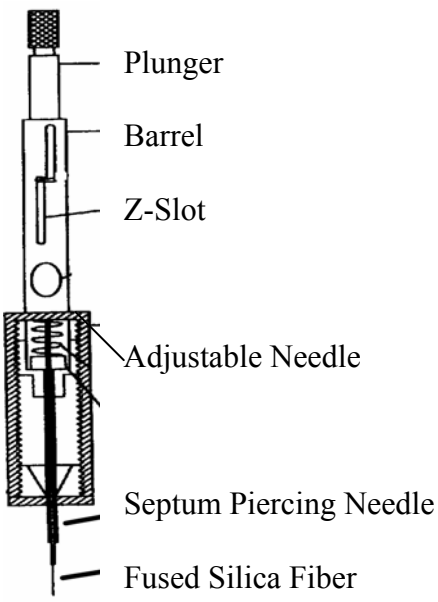

Figure 2. Schematic diagram of SPME assembly.

On-fiber derivatization of aldehydes and acetone with PFBHA

DMS/DVB (65 um) fiber was selected because it adsorbed PFBHA with greater reproducibility than the other fibers, such as 100 um PDMS.[24]

Figure 3 shows Diagrammatic procedures of on-fiber derivatization of aldehydes with PFBHA followed by GC/MS. At first, loading of PFBHA was performed by placing the PDMS-DVB fiber syringe into the headspace of PFBHA solution. 1ml of PFBHA solution (11.0 mg/ml) was introduced into an 8-ml headspace vial with a 1-cm magnetic stirring bar. A stirring ratio of 1100 rpm was used in the whole experiment. The fiber was exposed into the headspace at 20 ^{o}C for 10 min. Next, the fiber adsorbing

PFBHA was further exposed in the headspace of acetaldehyde calibration solution at 37 ^{o}C since the cell growth requires this exact temperature. Different exposure time (2, 5, 10, 15, 20, 30min.) was selected to figure out the optimum reaction time between acetaldehyde and PFBHA. Finally, the oximes formed on the fiber were desorbed at GC injector at a temperature of 250 ^{o}C for 2 min.

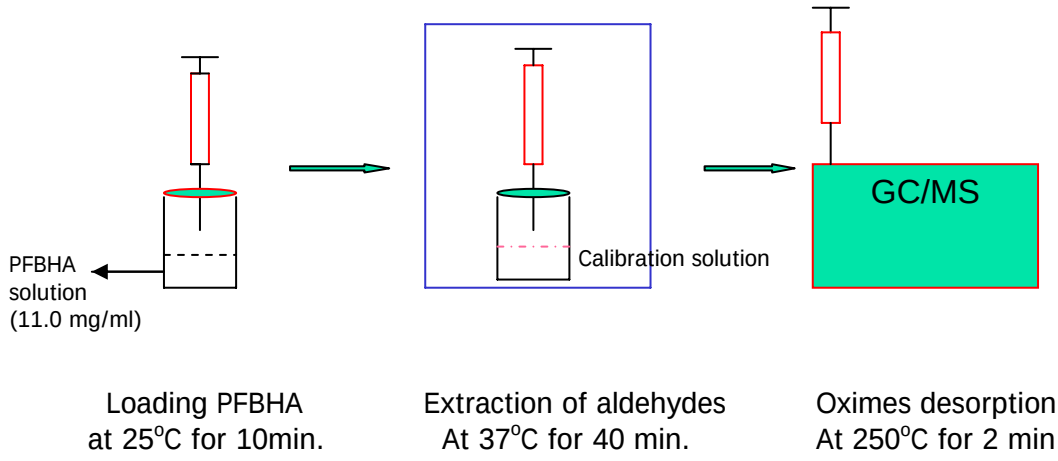

Figure 3. Diagrammatic procedures of on-fiber derivatization of aldehydes with PFBHA followed by GC/MS.

A special treatment was applied to acetone analysis to prevent contamination. Adsorption of PFBHA was performed directly in the headspace of chemical solid of PFBHA at room temperature of 20 ^{o}C for 10 min. All other procedures were performed under the same condition as acetaldehyde.

Validation of the method

The linear range of the method was investigated by determination calibration curves in the concentration range of interest. 15 ml aqueous solution containing acetaldehyde at concentrations of 0.5-20 uM was added to 15-ml vials. The line of best fit for the relationship between peak areas (obtaining by integrating the selected m/z 181 chromatograms) and concentrations of the analytes was determined by linear regression.

The precision of the method was expressed by the relative standard derivation (%, RSD). Four replicate analyses of a calibration solution containing 10 uM acetaldehyde were conducted in order to obtain the RSD values. From the calibration analysis of low concentration (500 nM), the detection limit was calculated on the basis of signal-to-noise (S/N) ratio =3.

Cell culture study

Two non-small lung cancer cells were studied. SK-MES-1 and NCI-H522, purchased from American Type Culture Collection (ATCC), were non-small cell lung cancer cell lines. Both cell lines were obtained from patients with histological diagnosis of non-small cell lung cancer. According to the provider's instruction, both cell lines were grown in a complete media. Table 1 lists the type and media for the two non-small cell lines. Cell growth was conducted in tissue culture flasks (Nunclon) at 37 ^{o}C in a 5% CO_2 incubator.

Table 1. Type and media of the cell lines studied.

Cell line	Type	Media and additives
SK-MES-1	Squamous cell carcinoma	Minimum essential medium 90% Fetal bovine serum 10%
NCI-H522	Adenocarcinoma	RPMI 1640 medium 90% Fetal bovine serum 10%

Figure 4 shows a diagrammatic flowchart of cell culture study using GC/MS and SPME with on-fiber derivatization. The Cell was detached from the tissue culture flasks before reaching confluence using trypsin/EDTA. Complete medium was then added and the cell with medium was spun in a centrifuge at 1200 rpm for 7 minutes. The supernatant was removed and new medium was added again. The cell numbers were counted by using the hemacytometer, then approximately 5, 15, 30, and 50 million cells in 15 ml of medium were split into four 40-ml autoclaved glass vials sealed with septum. The sealed vials with cell culture were placed in an incubator at 37 ^{o}C overnight for 16 hours together with a control vial that contained the same volume of medium without cells. The headspaces over the medium-only and the cell/medium cultures were allowed to develop overnight then were subjected to analysis by GC/MS and SPME with on-fiber devivatization.

Following the aldehydes analysis, the pH of each medium/cell was measured. Cell counting was performed again to determine the survivals rates in each cell/media culture.

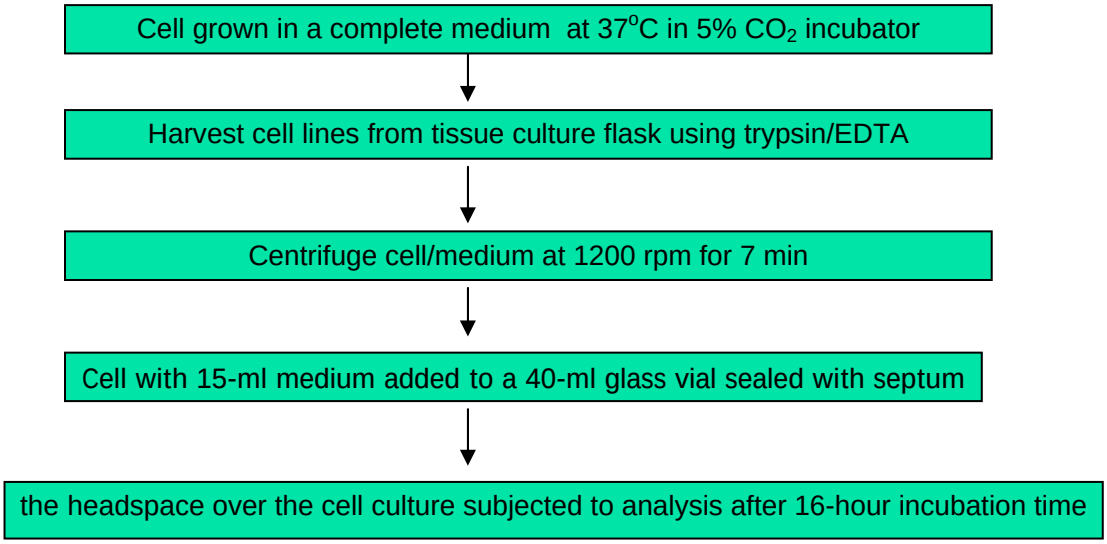

Figure 4. A diagrammatic flowchart of cell culture study using GC/MS and SPME with on-fiber derivatization**.**

RESULTS AND DISCUSSION

Optimization of on-fiber derivatization conditions

At the first step, adsorption of PFBHA was performed by exposure of the fiber in the headspace of 11 mg/ml PFBHA aqueous solution for 10 min. (stirring ratio of 1100 rpm, extraction temperature of 20^{o}C). The equilibrium time is around 15 min. [27] Because of low concentration of aldehydes in cell sample the mass loaded on the fiber in extraction time of 10 min is enough for derivatization of aldehydes. At temperature of 250 ^{o}C, the desorption efficiency of aldehyde oximes was found to be 99.96% when the desertion time was 2 min. [23] Therefore, SPME desorption was performed at 250^{o}C for 2 min.

Both temperature and time can affect fiber derivatization of aldehydes with PFBHA. Since cell sample requires culture temperature of 37^{o}C, the adsorption of aldehyde on fiber in all the tests was performed at this temperature in an incubator. Figure 5 shows the effect of extraction time on the amounts of aldehyde oximes. It can be seen that the oxime level dramatically increase with extraction time, and that the oxime level increased very slowly after 40 min. Therefore, 40 min. were selected as the aldehyde extraction time.

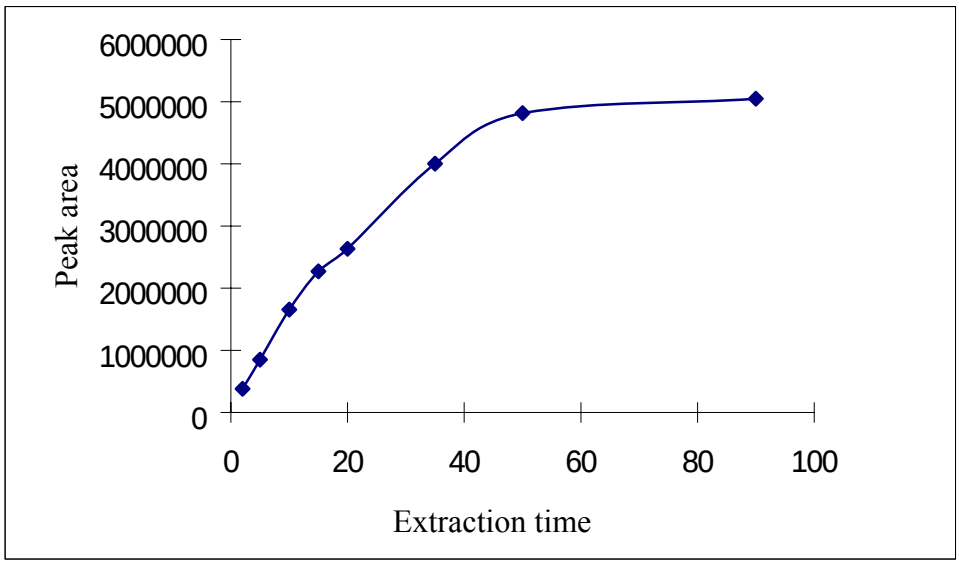

Figure 5. Adsorption-time profiles for acetaldehyde in a calibration solution (10 uM) using headspace SPME with on-fiber derivatization.

Validation of the method

Calibration curve of acetaldehyde is shown in Figure 6. There is a good relationship between the peak area and acetaldehyde concentration. The linear range of acetaldehyde is 100 nM-20 uM. Four replicate calibration solutions (5uM) were used to calculate the relative standard deviation (RSD) value which was less than 6% for acetaldehyde. The limit of detection (LOD) value is 50 nM for acetaldehyde with selected ion (*m/z* 181) monitoring mode. The wide linear range, low RSD and LOD values show that GC/MS and SPME with on-fiber derivatization is a reliable method for the analysis of aldehydes.

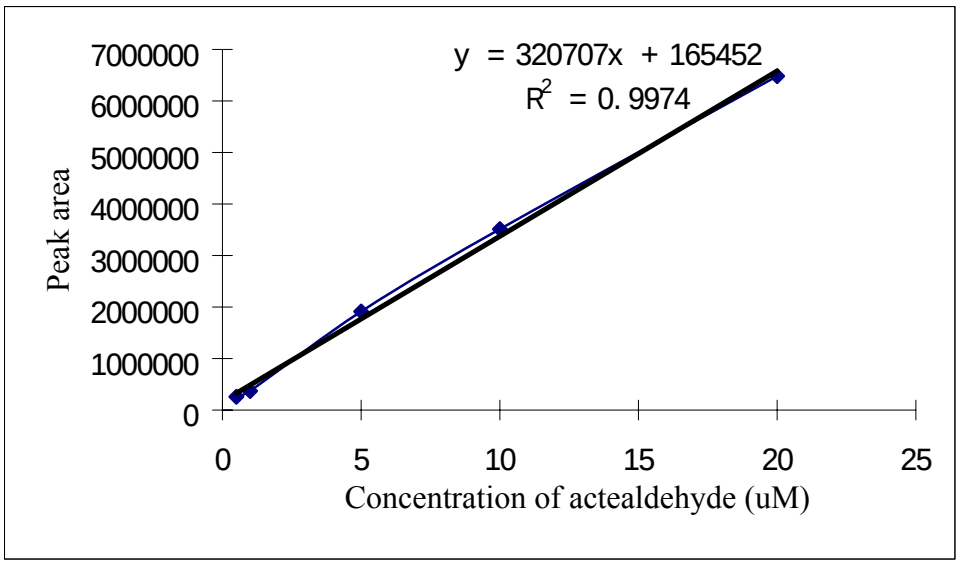

Figure 6. Calibration curve of acetaldehyde aqueous solution.

SK-MES-1 lung cancer cell study

The optimum adsorption conditions (37 ^{o}C and 20 min) were used for extraction of aldehydes in the cell culture sample. Figure 7 and Figure 8 show the selected ion monitoring (SIM) chromatogram of aldehydes in headspace of medium only and cancer cell with medium culture, respectively, by GC/MS and SPME with on-fiber derivatization. Formaldehyde, acetaldehyde and acetone were detected with current technique. The mass spectra of oximes of formaldehyde, acetaldehyde, and acetone is listed in Table 2, showing that each oxime has a base peak at *m/z* 181. This fragment ion originates from the pentafluorobenzyl moiety. This characteristic ion at *m/z* 181 was used in the SIM mode to determine the aldehyde in the cell culture with medium.

Table 2. Retention time (R.T.) and EI mass spectra data of PFBHA oximes

Oximes	R.T. (min)	Base Peak (*m/z*)	Other main ions(*m/z*)
Formaldehyde	8.88	181	195, 225
Acetaldehyde (*syn*)	10.17	181	195, 209, 239
Acetaldehyde (*anti*)	10.29	181	195, 209, 239
Acetone	10.84	181	195, 223, 253

From Figure 7 and Figure 8, we can see that acetaldehyde concentration in the headspace of cell culture decreased compared to that of the medium. Using calibration curve of an external standard method, the acetaldehyde level in the medium was quantitatively analyzed. The concentration of acetaldehyde in the medium was 0.5 μM, while it decreased to an undetectable level in the cell culture after 16-hour incubation time. Five replicate tests with same number of cells were performed to confirm this conclusion. Different number of cells in culture medium was also studied but with the same results. The cell survival rates for SK-MES-1 cell were around 60%. The pH of the SK-MES-1cell culture and medium decreased from the pre-incubation value of 7.95 towards acidic levels at the end of the test (about 16-hour incubation time).

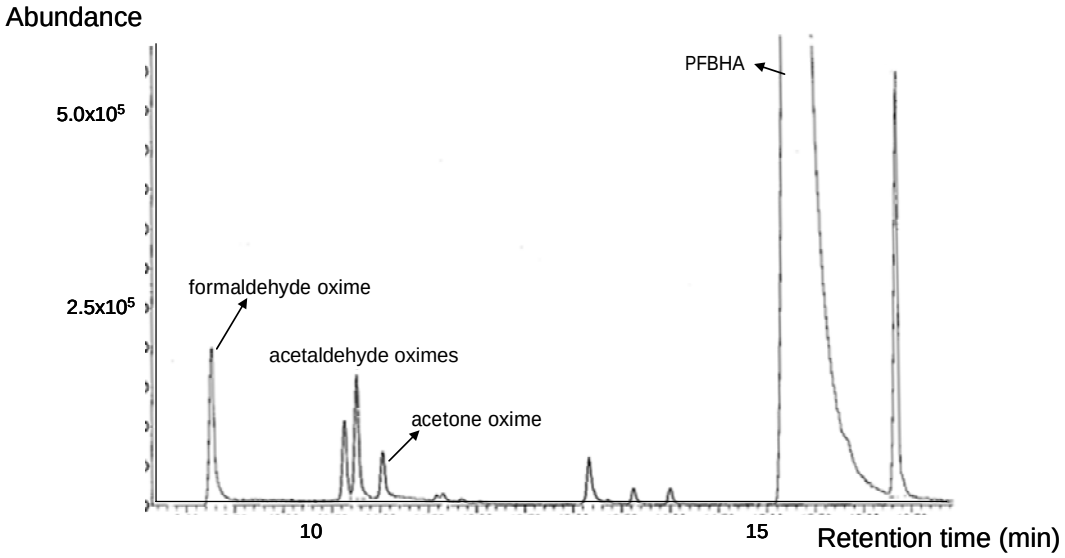

Figure 7. The GC/MS SIM (*m/z*) chromatogram of medium by SPME with on-fiber derivatization.

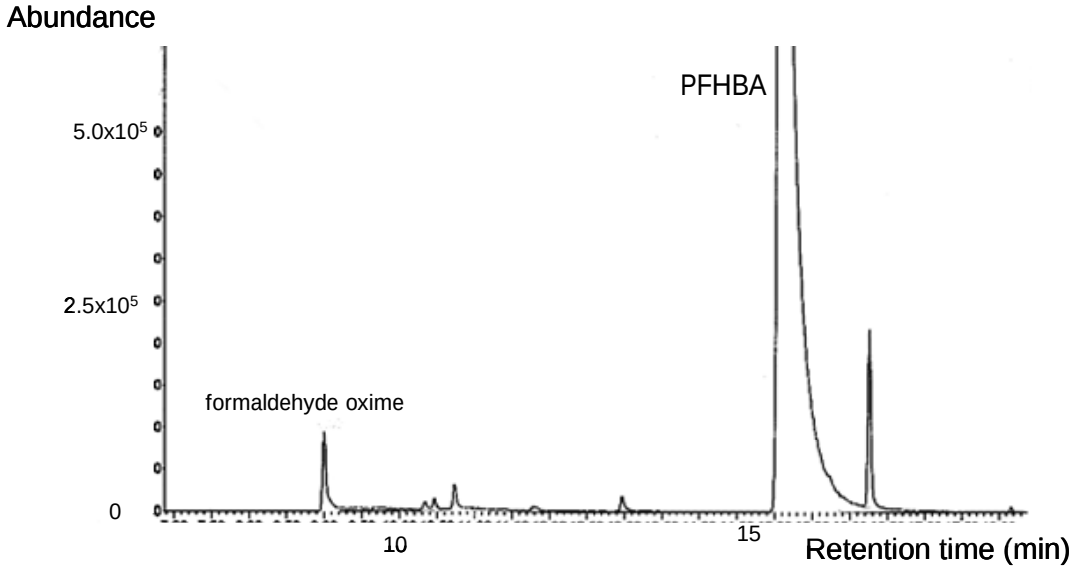

Figure 8. The GC/MS SIM (*m/z*) chromatogram of cell culture with medium by SPME with on-fiber derivatization.

An interesting experiment was performed concerning the decrease of acetaldehyde concentration in the cell culture. Different concentration of acetaldehyde solution in methanol, including 0.5 μM, 1 μM, 2 μM and 5 μM, was spiked into the cell culture with 5 millions cells together with a control medium. The cell culture and control medium were allowed to stay around 16 hours before subjected to GC/MS with SPME analysis. Figure 9 shows the diagram of spiking cell culture with acetaldehyde solution. As to the first three acetaldehyde spiking (0.5 μM, 1 μM, and 2 μM), the concentration of acetaldehyde were found to be at an undetectable level which means the acetaldehyde concentration was less than 0.05 uM. For the 5 uM spiking, the concentration of acetaldehyde decreased to 1.0 uM after 16-hour incubation time. Since there was 0.5 uM acetaldehyde in the control medium, therefore 5 million of SK-MES-1 cells could consume 4.5 uM acetaldehyde in 15-ml medium over a 16-hour period at 37 ^{o}C. Figure 10 and Figure 11 below showed the SIM chromatogram of medium control and cell culture after spiking 2 μM of acetaldehyde, respectively. By comparing the chromatogram of the two figures, it was clear that the cell culture consumed almost all the spiking acetaldehyde (Figure 10) while there was a large scale increase of acetaldehyde concentration in the control medium (Figure 11) compare to Figure 7- the SIM chromatogram of medium without spiking acetaldehyde.

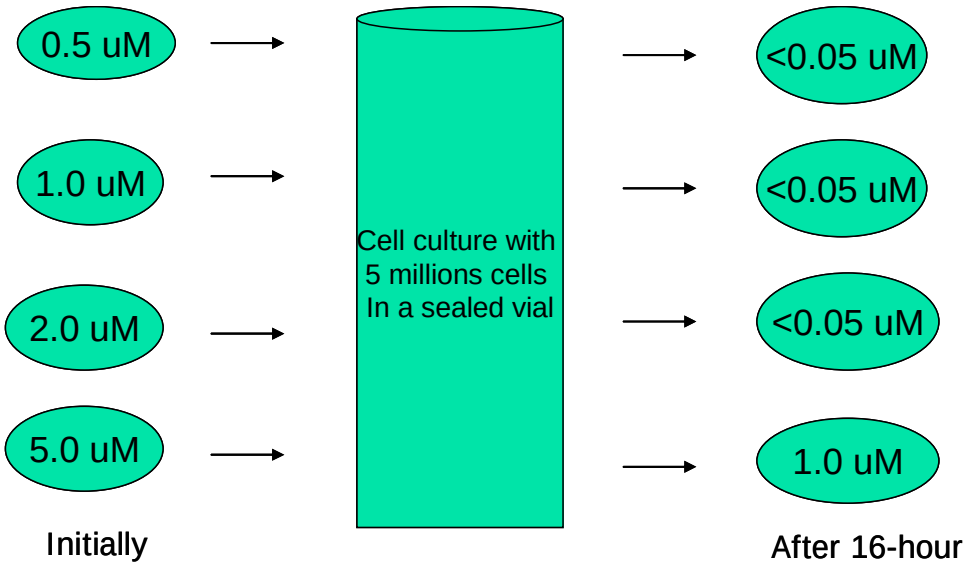

Figure 9. The diagram of spiking cell culture with acetaldehyde solution.

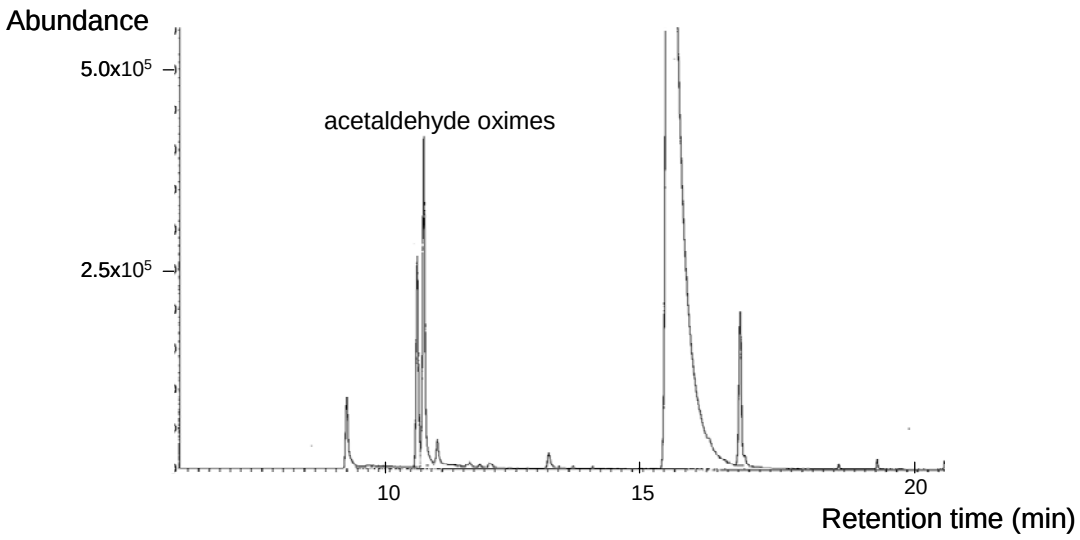

Figure 10. The GC/MS SIM (*m/z*) chromatogram of medium with spiking of acetaldehyde (2 uM) by SPME with on-fiber derivatization.

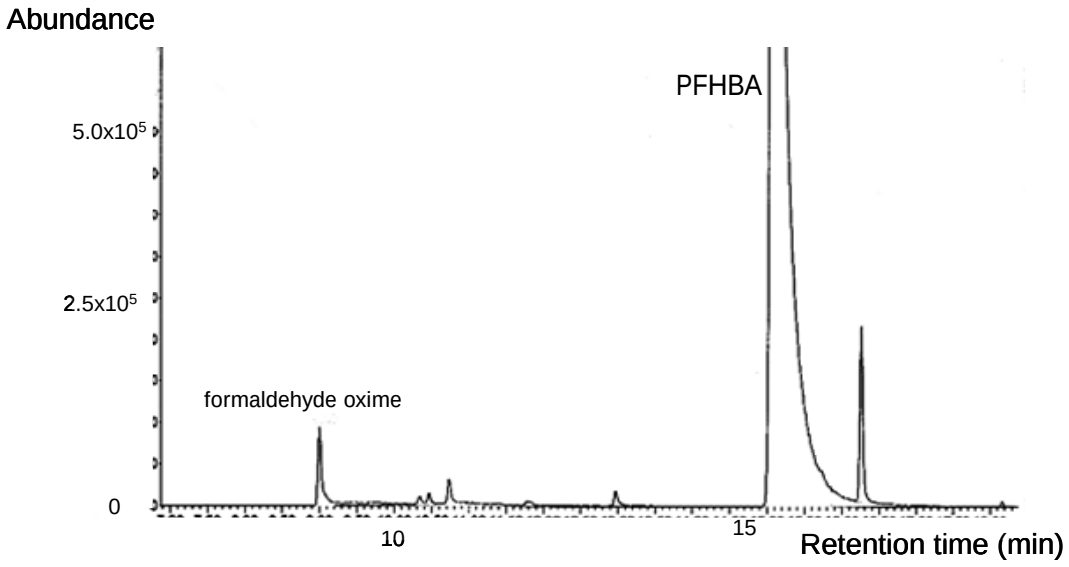

Figure 11. The GC/MS SIM (*m/z*) chromatogram of cell culture (5 millions cells) with spiking of acetaldehyde (2 uM) by SPME with on-fiber derivatization.

NCI-H522 lung cancer cell study

NCI-H522 lung cancer cell which is an adenocarcinoma non-small lung cancer were also studied using GC/MS Spectrometry and SPME with on-fiber derivatization. The results of NCI-H522 cell study were very similar to those of SK-MES-1 study. Same aldehydes were detected in SIM chromatogram of cell culture study including formaldehyde, acetaldehyde and acetone. The acetaldehyde concentration of cell culture decreased from 1.0uM to an undetectable level after 16-hour incubation time. The spiking of acetaldehyde experiments showed that 5 million NCI-H522 cells could consume 5.9 uM acetaldehyde.

Table 3. Comparison of two non-small lung cancer cell studies.

Cell culture (5 millions cells)	pH change after 16 hours	Survival rate	Acetaldehyde consumption
SK-MES-1 (Squamous cell Carcinoma)	7.95 ~ 7.65	~70%	4.5 uM
NCI-H522 (Adenocarcinoma)	7.31 ~ 6.80	~50%	5.9 uM

Discussion

Table 3 lists the comparison results of two non-small lung cancer cell studies. The experiments showed that SK-MES-1 and NCI-H522 cell lines both consumed acetaldehyde molecules from the medium and spiking acetaldehyde solution. Therefore acetaldehyde can be considered as a molecular cancer marker for lung cancer. Smith *et al*. reported acetaldehyde enhancement in the headspace of SK-MES-1 cell culture using selected ion flow tube mass spectrometry (SIFT-MS), and the concentration found to be proportional to the number of cancer cell in the medium. [16] In Smith's study, he also claimed that there was relatively large background of ethanol from the medium. Miekisch *et al*. discussed in a review about acetaldehyde source of human breath and said that it was probably produced by oxidation of endogenous ethanol. [28] So the acetaldehyde enhancement what smith claimed might come from the ethanol because the headspace air over the cell culture was heated before introducing to the SIFT-MS instrument. GC/MS with on-fiber derivatization technique had a low detection limit of

acetaldehyde at 50 nM. The concentration enhancement was able to be found if a certain number of cells had given off a certain amount of acetaldehyde. Since the extraction of aldehydes from the headspace of cell culture in this study was performed at 37 ^{o}C, there was no interference of headspace air over the cell culture. The amount of acetaldehyde in the medium may be consumed in the metabolism of lung cancer cells. Acetaldehyde has multiple mutagenic effects.[29-31] It is carcinogen in animal models[32] and a suspected human carcinogen.[33] Recent study about biochemical reactions linking alcohol consumption to cancer suggested that cellular polyamines facilitate the formation of crotonaldehyde (CrA) from acetaldehyde. CrA then reacts with DNA to form the mutagenic adduct α-methyl-γ-hydroxy-1, N2-propano-2'-deoxyguanosine (Cr-PdG).[34] Figure 12 shows the mechanism of acetaldehyde-derived DNA adduct formation and the proposed relationship of Cr-PdG adducts to the known cytogenetic effects of acetaldehyde.[34] Another explanation about consumption of acetaldehyde is that it is metabolized to acetate by aldehyde dehydrogenases inside the cell line.[35]

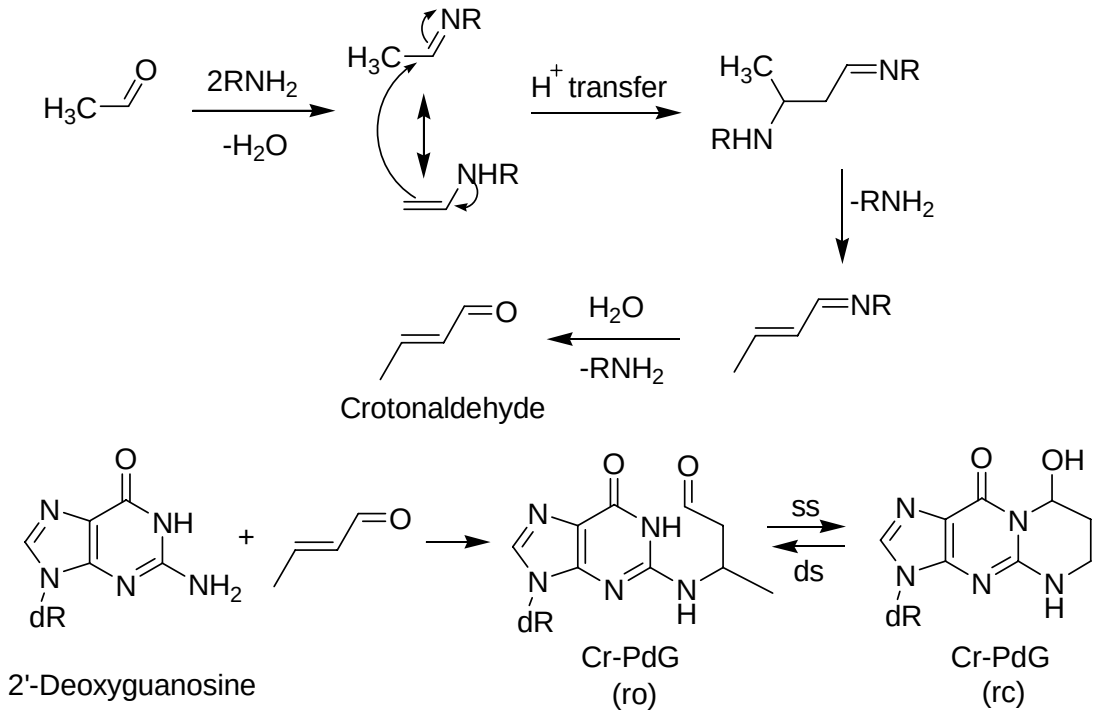

Figure 12. Mechanism of acetaldehyde-derived DNA adduct formation and the proposed relationship of Cr-PdG adducts to the known cytogenetic effects of acetaldehyde. The ring-opened (ro) and ring-closed (rc) forms of the Cr-PdG adducts are shown. Other abbreviations: ss, single-stranded DNA; ds, double-stranded DNA.

There might be a little concentration change of formaldehyde and acetone between the medium only and cell culture with medium. The experiment data showed some of tests with a little difference while others without. To figure out whether there was a change or not, further experiments were performed.

Low molecular weight carbonyls, including formaldehyde, are highly volatile and reactive. It is well known that formaldehyde is ubiquitous in the environment and is commonly found in air, water and industrial products. Two blank tests were run after exposing PDMS/DVB fiber into the headspace over both PFBHA solution and solid PFBHA. The chromatogram showed that there was a high peak of formaldehyde oxime in both of the two blank PFBHA tests. The signal of formaldehyde oxime of blank PFHBA was very close to that of cell culture and medium. This proved the formaldehyde came from the aqueous solution of PFBHA and there was no concentration change between cell culture and medium. The detection limit was unable to be determined with the current technique.

A former student who recently graduated from our group claimed acetone enhancement in the cell culture of SK-MES-1 by using purge and trap and GC/MS in her thesis.[26] The problem for acetone is that there was always certain amount of acetone in the double distilled water used to prepare the aqueous solution of PFBHA and acetone. In order to eliminate the contamination the fiber was placed in the headspace over solid instead of aqueous solution of PFBHA. There was no acetone oxime peak in the blank fiber test and following experiments data showed that there were no detectable acetone in both cell culture and medium. The detection limit for acetone was 1 uM.

SUMMARY

A GC/MS method with SPME and on-fiber derivatization was developed for the detection of aldehydes in lung cancer cell culture. It has been demonstrated that this method is a simple, repaid, sensitive and solvent-free technique for analysis of aldehydes in lung cancer cell culture. Using this method, acetaldehyde decrease was found in both of non-small lung cancer cell culture studied compared to the medium control study. The results of spiking the cell culture with acetaldehyde solution showed that 5 million SK-MES-1 cell lines could consume up to 4.5 uM acetaldehyde in 15-ml medium, and 5 million NCI-H522 cell lines could consume 5.9 uM acetaldehyde in 15-ml medium. Acetaldehyde might be consumed in the metabolism of lung cancer cell. One possible mechanism to explain the acetaldehyde consumption is that acetaldehyde converted to CrA by polyamines in dividing cells, forming Cr-PdG, which is mutagenic.

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