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Variation in Volatile Organic Compound Content in Normal Human Breath

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Abstract: **Analysis of volatile metabolites in a normal or healthy human breath was successfully carried out using a novel approach with an analytical technique that is reproducible and highly sensitive. The goal of this preliminary study was to determine whether in normal human breath intra-individual variation exist in volatile organic compound profiles. Active SPME GCMS was successfully employed to achieve the goal of this study. The results obtained indicate that in breath analysis there is significant natural intra-individual variation in VOCs profiles. Several compounds were detected in all samples collected throughout the day, however, only few were consistently present. Small peak areas from the chromatograms indicate that many of the VOCs in the breath were present in trace concentrations. In male breath, the number of VOCs detected was higher in morning (8a.m) samples and decreased throughout the day whiles in female; the afternoon (2 p.m) samples contained the greater number of VOCs. The chemicals, dimethyl ether, D-limonene, and isopropyl alcohol were present consistently in male breath with D-limonene and isopropyl alcohol present at low concentrations. A high relative standard deviation (0.17-0.72) in VOCs peak areas is an indication that concentration of these compounds changed significantly throughout a typical day. The change in concentrations of dimethyl ether throughout the day was not consistent but that of D-limonene decreased throughout the day whiles that of isopropyl alcohol was high in the morning and remained fairly constant for the rest of the day.**

Keywords: **Metabolites, reproducible, sensitive, intra-individual, VOCs, active SPME GCMS, chromatograms, relative standard deviation.**

I. INTRODUCTION

Biomarkers are set of volatile metabolites produced by an organism that could be used for identification of the organism or identification of some biological and pathogenic processes within the organism. The use of volatile biomarkers for health diagnosis may one day become attractive because of its noninvasive, low cost per analysis, user-friendly and fast method for disease diagnosis and therapeutic monitoring compared to other biomarker screening methods that involve the collection and analysis of blood, urine, and stool samples [1]. This method is convenient for both critically ill patients and small children. It has been established that human breath contains hundreds of volatile organic compounds (VOCs) that could be used as a chemical signature of certain diseases [2]. Breath analysis has proven to be a useful diagnostic tool for diseases such as diabetes [3], lung disorders [4], gastrointestinal and liver disease [5], and lung cancer [6]. The abnormal levels of breath chemicals such as isoprene, acetone, pentane, and nitrotyrosine have been associated with metabolic disorders and diseases such as elevated blood cholesterol, lung cancer, liver diseases; and asthma respectively [7].

The major limitations of breath analysis are lack of standard sample collection protocols and analysis methods [8], [9]. Many studies have been done in this field without good agreement because of the differences in sample collection and analysis methods [10]. Subject health, diet, and endogenous and exogenous factors could also influence the VOC profiles. Volatile organic compounds are constituent of blood volatile metabolites and have concentrations related to those in the blood. Volatile organic compounds diffuse easily from the blood into the air due to the large surface area and tiny size of the alveoli [11]. Separating alveoli breath and those in the upper airways (mouth/pharynx) is challenging in breath

analysis. The air in the upper airways does not take part in the gas exchange with the blood and therefore VOCs present are considered exogenous chemicals [12]. The viability of using human breath for health analysis relies on the fact that there is a physiological gas exchange between air and blood across the pulmonary alveolar membrane. In this work an attempt was made to reduce the interferences of VOCs in upper airways with that in breath by rinsing the mouth three times with water and flushing the mouth and the nasal pathways three times with normal inhaled and exhaled breath prior to breathe collection. VOCs in human breath originate from metabolic activities in the body and have the potential to be used as disease biomarkers and also to determine the state of health or metabolic disorders of patients. In breath analysis, alveoli gradient could also be useful in eliminating any possible exogenous chemicals with positive alveoli gradient indicating the presence of an endogenous chemical [9].

There are two main approaches for selecting VOC biomarkers of diseases. The first approach, which is said to be pathogen-specific, requires that single or multiple biomarkers of certain diseases appear frequently in the breath of the patient suffering from the pathogen related affliction. It is important also to emphasize that some unique biomarkers may change concentration during internal metabolic processes and this change plays a significant role in therapeutic disease monitoring. The second approach takes into account both pathogen-specific biomarkers and those generated in vivo during host-pathogen interactions [13].

II. MATERIALS AND METHODS

A. Breath sampling and analysis:

In sample collection, the mouths of male and female human beings were rinsed three times with normal drinking water to eliminate interferences of food traces and odor in the mouth. The mouth and the nasal pathway were also flushed three times with normal inhalation and exhalation to eliminate interferences of exogenous chemicals. A male and a female inhaled and held their breath for 30 s before exhaling into a Teflon collection bag (minimum 850 ml). Samples were collected prior to eating while at rest (no food consumption or strenuous physical activity for at least 60 min prior to sample collection). Teflon bags were selected over commercially available sample bags because of their chemical inertness and thermal stability. In both male and female breath samples were collected from 8 a.m to 8 p.m at 3 hours intervals for six days.

Breath samples of volume (850 ± 0.5) ml were preconcentrated by an Entech 7150 concentrator. In active SPME, the preconcentration utilizes three cold traps: (1) Tenax (2, 6-diphenylene oxide) (lighter VOCs trap), (2) solid phase micro extraction (SPME) (heavier VOCs trap) (PDMS), and (3) (water trap) (lighter VOCs). Before VOCs get to the GC column for separation, the five steps outlined in Table 1 and in appendix (figure 6) were followed. After the preconcentration step, the VOCs were separated utilizing an Agilent GC equipped with a DBI column. A temperature program utilized is as follows: 35 ºC for 5 min, 4 ºC/min to 110ºC then hold for 1 min, 15 ºC/min to 220 ºC for 5 min. Mass spectrometric analysis was done using a 5975C triple axis mass detector at a scan speed of 4.3 Hz from 45 to 206 m/z mass range. Four internal standards IS1 (bromochlorobenzene), IS2 (1, 4-difluorobenzene), IS3 (chlorobenzene-d5), and IS4 (1-bromo-4-fluorobenezene) were injected automatically into the Entech preconcentator

Agilent chemstation software was employed to process the data. The compounds present were identified using the NIST 2008 GCMS library. The retention time and peak areas were standardized using the four internal standards used in this study.

TABLE 1: Instrument trap time and temperature conditions for the preconcentration steps

Steps	Time (min)	Event	Time (min)	T1 (^{o}C)	T2 (^{o}C)	T3 (^{o}C)	Flow rate (ml/min)
1	15	Trapping	15	50	-40	-50	55
2	2	Recover	2	50	0	-50	10
3	2	Bake out	2	-52	70	-50	25
4	2.8	Refocus	2.8	-52	50	200	25
5	6	Injection	6	230	160	200	2

T1= SPME trap temperatures (heavier VOCs trap in T1), T2= water trap temperatures (lighter VOCs trap in T2), T3= Tenax trap temperatures (lighter VOCs trap in T3)

III. RESULTS AND DISCUSSIONS

Several peaks observed in the chromatograms is an indication that in human breath many volatile metabolites are present; and also the small peaks areas suggest that most compounds were present in low concentrations. More than thousand different VOCs have been detected previously in normal human breath by Shneh et.al [14]. Low concentrations of important breath VOCs observed in this study suggest that breath analysis requires highly sensitive analytical instrumentation for accurate detection and quantification [15]. The chromatograms of some samples collected in this study are shown in appendix (figure 7). A significant variation in breath VOC profiles from the same subject was observed in samples taken only 3 hours apart. This implies that in human breath significant intra-individual variation in VOC profiles exist. The intra-individual variation in breath volatile metabolites makes this method difficult for disease diagnosis and therapeutic monitoring despite the numerous advantages.

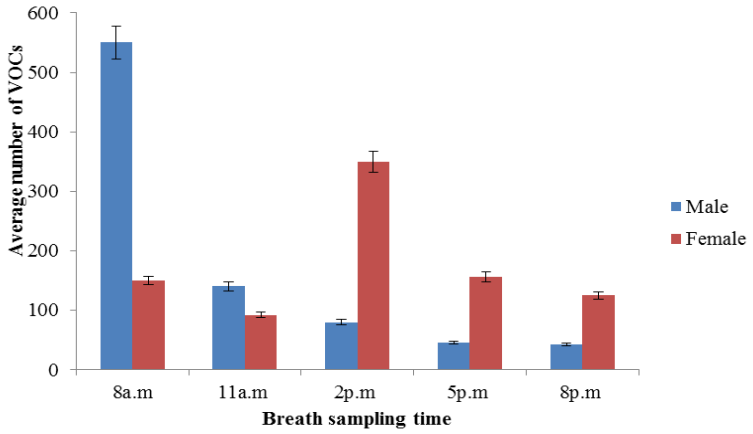

Fig 1: Average number of VOCs present in male and female breath collected at 3 hours intervals throughout the day for six replicate days. Error bars indicate the percentage standard deviation.

In male breath the number of VOCs detected was higher in morning samples (8a.m) and decreased throughout the day (figure 1). In female breath the number of VOCs detected throughout the day does not follow a particular trend. Several VOCs were detected in afternoon samples (2p.m) (figure 1). The reason could be that in male breath many of the VOCs may still be present throughout the day but their concentrations decreased to a point below the limit of detection of the analytical instrument. The difference could have resulted in the inconsistencies in VOC concentration profiles (figure 2).

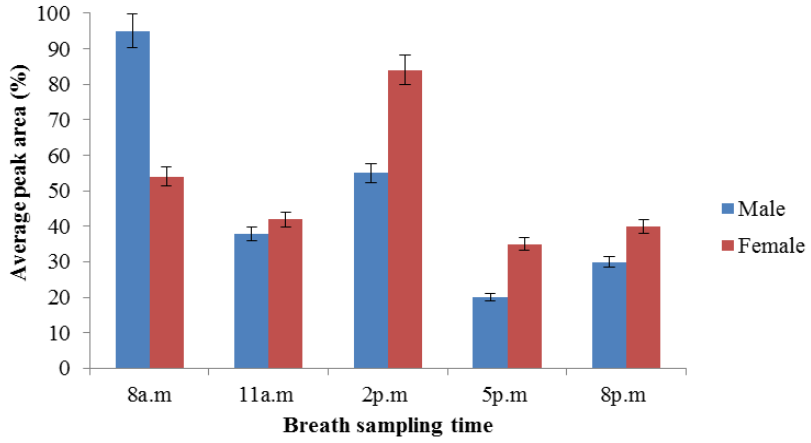

Fig 2: Concentration profiles of VOCs present in male and female breath collected at 3 hours intervals for six replicate days. Error bars indicate the percentage standard deviation.

Even though an attempt was made to reduce the interferences of VOCs in the upper airways with that in human breath, VOCs in ambient air could have possibly impacted the VOC profiles to some extent. Differences in food consumption and physical activity levels associated with a typical could have also lead to the observed differences in breath VOCs.

In male breath, the chemicals, dimethyl ether, D-limonene and isopropyl alcohol were present consistently in breath samples collected throughout the day. The change in concentration profiles of these chemicals was monitored in this study.

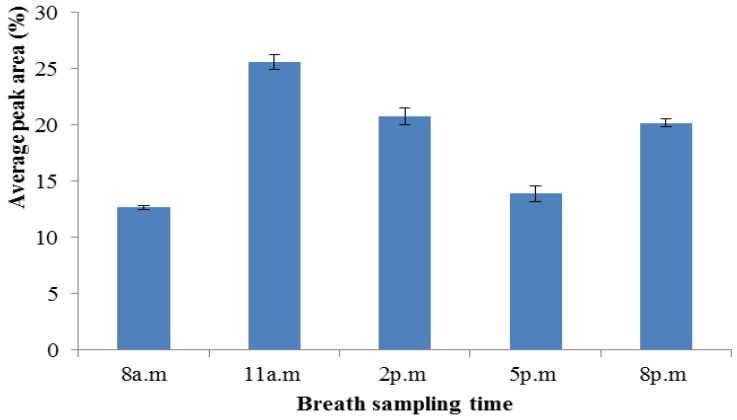

Fig 3: Concentration profiles of dimethyl ether in male breath. Error bars are standard deviation of 6 replicate days.

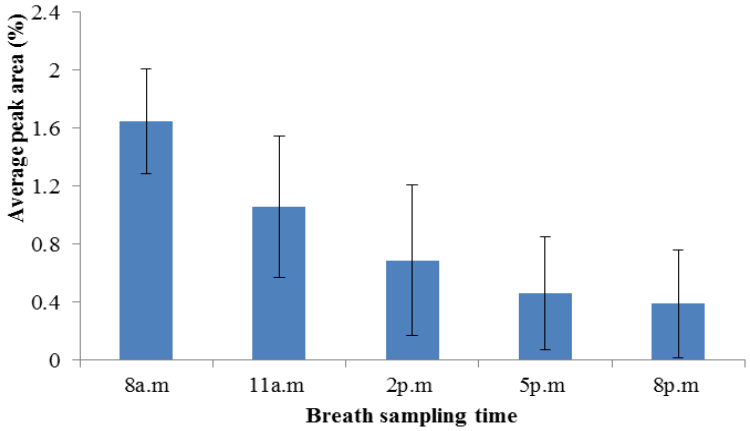

Fig 4: Concentration profiles of D-limonene in male breath. Error bars are standard deviation of 6 replicate days.

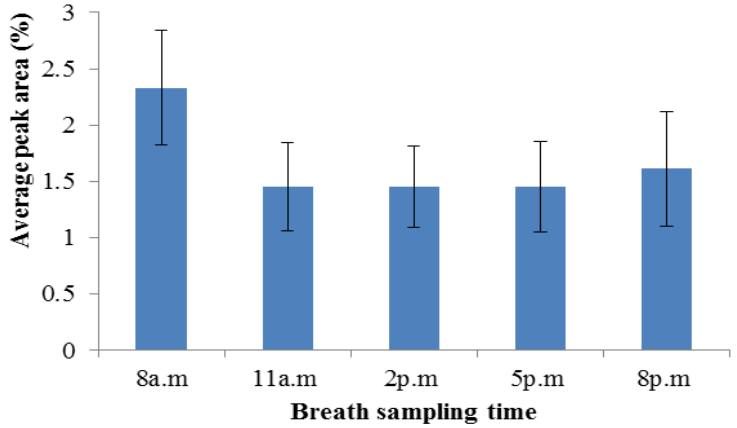

Fig 5: Concentration profiles of isopropyl alcohol in male breath. Error bars are standard deviation of 6 replicate days.

The D-limonene and isopropyl alcohol were present at low concentrations compared to dimethyl ethyl. The change in dimethyl ether concentration was not consistent; the D-limonene concentration decreased throughout the day and the concentration profiles of isopropyl alcohol was high in the morning and remained fairly constant throughout the rest of the day as shown in figure 3, figure 4, and figure 5 respectively.

IV. CONCLUSION

Active SPME-GCMS was successfully employed to detect VOCs in the breath of a normal male and female. In both male and female breath, a significant natural intra-individual variation in the VOC profiles exists. This makes health diagnosis from breath VOC profiles extremely challenging. In breath samples, most chemicals were present at low concentrations and thus a preconcentration step, such as active SPME, can aid in sample analysis. Most chemicals were not consistent in the breath samples but dimethyl ether, D-limonene, and isopropyl alcohol was found common in male breath. The dimethyl ether concentration profiles were not consistent but that of D-limonene and isopropyl alcohol showed some trend in concentration profiles. Ambient air can also contribute to breath chemicals and all exogenous chemicals need to be identified in order to make biomarker identification more reliable. Principal component analysis will also be used in a follow-up studies to enhance the determination and selection of biomarkers of certain diseases.

V. APPENDIX

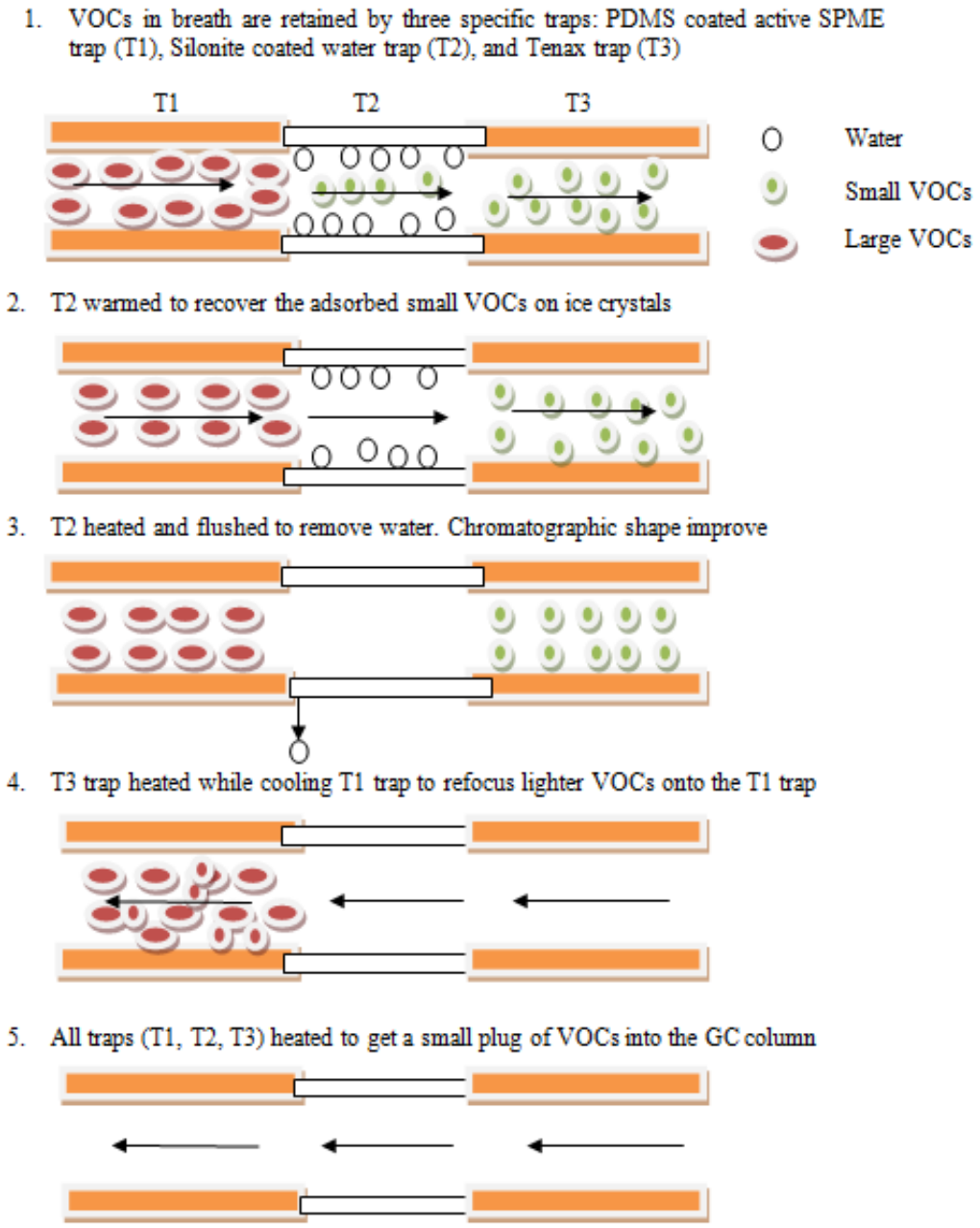

Fig 6: The five major steps used in preconcentration of breathe samples

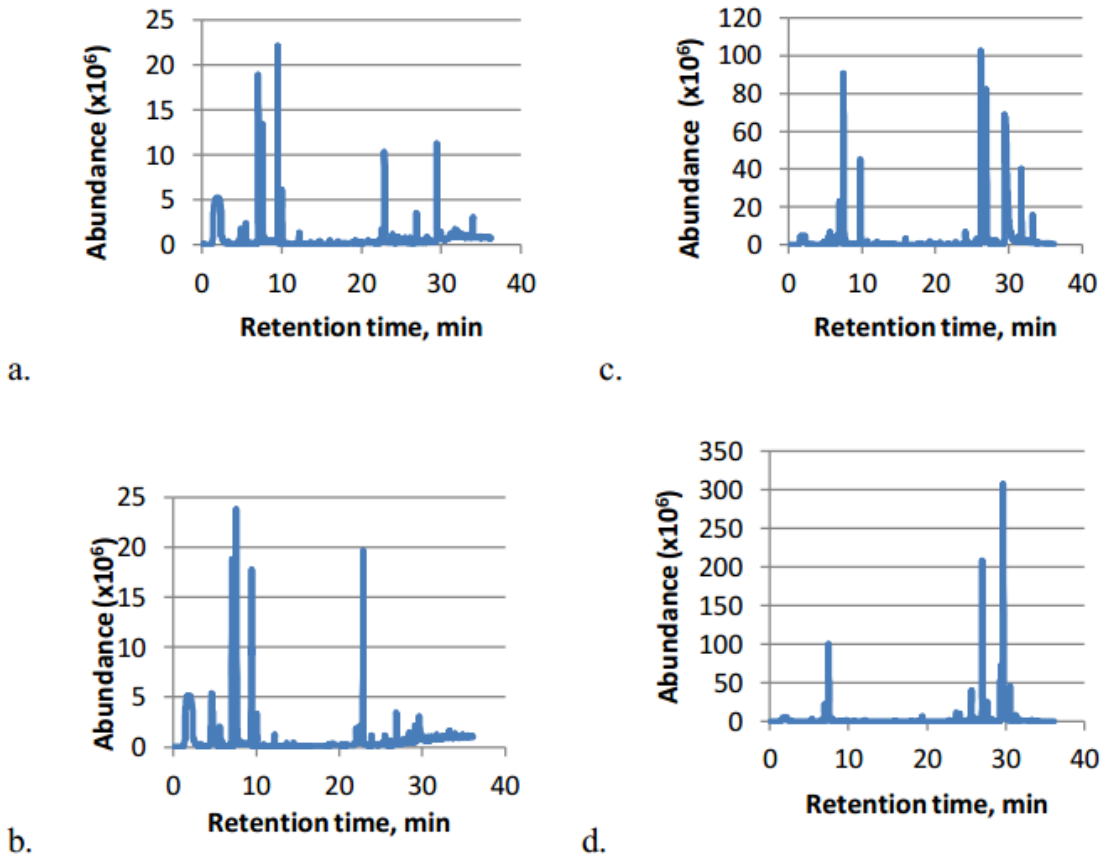

Fig 7: GCMS chromatograms of volatile metabolites in human breath: (a) Male breath at 8a.m, (b) Male breath at 11a.m, (c) Female breath at 8a.m, (d) female breath at 11a.m

REFERENCES

[1] S. Birgitta, W. Fredrik, V. Anders, "Analysis of breath samples for lung cancer survival". *Anal. Chim. Acta*, 2014, 840, pp.82–86.

[2] F.D. Francesco, R. Fuoco, M.G. Trivella, A. Ceccarini, "Breath analysis: trends in techniques and clinical applications". *Microchemical J.* 2005, 79, pp.405-410.

[3] M. Phillips, R.N. Cataneo, T. Cheema, J. Greenberg, "Increased breath biomarkers of oxidative stress in diabetes mellitus". *Clin. Chim. Acta*, 2004, pp.189-194.

[4] A.W. Boots, J.J.B.N. Van Berkel, J.W. Dallinga, A. Smolinska, M.F.M. Wouters, F.J. van Schooten, "The versatile use of exhaled volatile organic compounds in health and disease". *J. Breath Res*, 2012, 6, pp.1-21.

[5] C.S.J. Probert, I. Ahmed, T. Khalid, E. Johnson, S. Smith, N. Ratcliffe, "Volatile organic compounds as diagnostic biomarkers in gastrointestinal and liver diseases". *J. Gastrointestin. Liver Dis.* 2009, 18, pp.337-343.

[6] M. Hakim, Y.Y. Broza, O. Barash, N. Peled, M. Phillips, A, Amann, H. Haick, "Volatile organic compounds of lung cancer and possible biochemical pathways". *Chem. Rev.* 2012, 112, pp.5949-5966.

[7] W. Chuji, S. Peeyush, Breath analysis using laser spectroscopic techniques: Breath biomarkers, spectral fingerprints, and detection limits. *Sensors*, 2009, 9, 8230-8262.

[8] M.M. Gokhan, W.G. Kelvin, A.R. Richard, H.D. Larry, R. Isreal, "Collection and analysis of exhaled breath condensate in humans". *AJRCCM*, 2001, 164, pp.731-737.

[9] S. Jessy, J. Digambar, "Breath biomarker for clinical diagnosis and different analysis technique". *RJPBCS*, 2010, pp.639-653

[10] I. Horvath, J. Hunt, P.J. Barnes. "Exhaled breath condensate: methodological recommendations and unresolved questions". *Eur. Respir. J.* 2005, 26, pp.523-548.

[11] M. Hakim, Y.Y. Broza, O. Barash N. Peled, M. Phillips, A. Amann, H. Haick, "Volatile organic compounds of lung cancer and possible biochemical pathways". *Chem. Rev.* 2012, 112, pp.5949-5966.

[12] M. Phillips, R.N. Cataneo, A.R. Cummin, A.J. Gagliardi, K. Gleeson, J. Greenberg, R.A. Maxfield, and W.N. Rom, "Detection of lung cancer with volatile markers in the breath". *Chest*, 2013, 6, pp.2115-2123.

[13] M.M.L. Steeghs, S.M. Cristescu, P. Munnik, P. Zanen, F.J.M. Harren, "An off-line breath sampling and analysis method suitable for large screening studies". *Physiol. Meas*. 2007, 28, pp.503-514.

[14] S. Shneh, N. Ranjan, C. Trinad, "Clinical applications of volatile organic compound analysis for detecting infectious diseases". *Clin. Microbiol. Rev*, 2013, 26, 462-475.

[15] S. David, W. Tianshu, S. Josep, S. Patrik, E. H. Alicia, "Quantification of acetaldehyde released by lung cancer cells in vitro using selected ion flow tube mass spectrometry". *RCM*, 2003, *17*, 845-850.