Investigation on Volatile Organic Compounds in Breath.

Investigation into whether the concentration of VOCs in breath stored in Glass syringes decreases over time.

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The breathe team in molecular physics are currently trying to develop a test which can diagnose liver disease. They have found that the time taken from when samples of breath are first collected and then analysed can take up to 3 hours. This could change the concentration of VOC's within the sample. The effect of time on the concentration of VOCs present in the sample of breath taken was investigated. The investigation was carried out to consider whether leaving a syringe containing a sample of breath for different periods of time would affect the number and type of VOCs present. Breath samples were taken from two different healthy volunteers to allow comparison. Both sets of breath samples were taken in syringes then one set of breath samples was stored in an incubator at 24°C whilst the other set was stored in paper bags lined with bubble wrap. Subject A's breath samples were stored in the Incubator and subject B's breath samples in the paper bags. Each breath sample was analysed with a time interval of 30 minutes between them. The results did not prove nor disprove the hypothesis so in order to reach a conclusion, further studies will need to be carried out.

Investigation into whether the amount of VOCs in breath stored in Glass syringes decreases over time.

Introduction:

VOCs are carbon based chemicals which evaporate easily at room temperature. They can originate from plant and animal emissions or from motor vehicles and factories. Numerous products within our homes also release VOCs such as carpets, air fresheners and cosmetics. Several VOCs are present in human breath. The breathe team are currently trying to develop a test which can indicate whether or not a person has liver disease. Certain VOCs are endogenous meaning they are formed within the human body due to a person's metabolism. VOCs are thought to be biomarkers from which liver disease may be identified. The liver is a major metabolising organ and therefore VOCs present on the liver can allow the detection of liver disease.

When breath samples are collected from patients the time taken for them to be brought back to the lab and analysed can be up to almost 3 hours. This time is suspected to have an effect on the number of VOCs present. Therefore the results showing the amount of each VOC in the breath are no longer accurate. The test will not correctly diagnose patients if this is the case.

Aim:

The aim of this investigation was to find out whether storing the samples of breath in different places for a set period of time has an effect on the concentration and type of VOCs present within a sample.

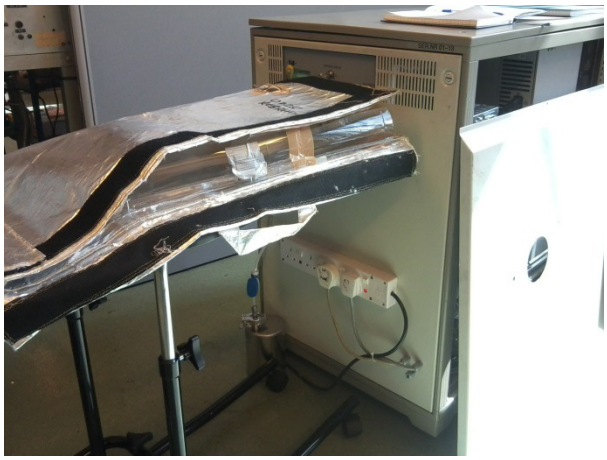
Hypothesis:

The concentration of Volatile Organic Compounds within a sample of breath decreases over time.

Method:

Instrumentation:

The PTR-MS (Proton Transfer Reaction Mass Spectrometer) is an incredibly sensitive instrument used to monitor the amount of VOCs (Volatile Organic Compounds) present in a sample of air. It uses hydronium ions to ionise the VOC's. By transferring one hydrogen ion from the hydronium ion it converts the hydronium ion into water. This only occurs in the presence of a VOC as H+ has a higher binding affinity to VOCs than water or ambient air. The molecules are then analysed as they

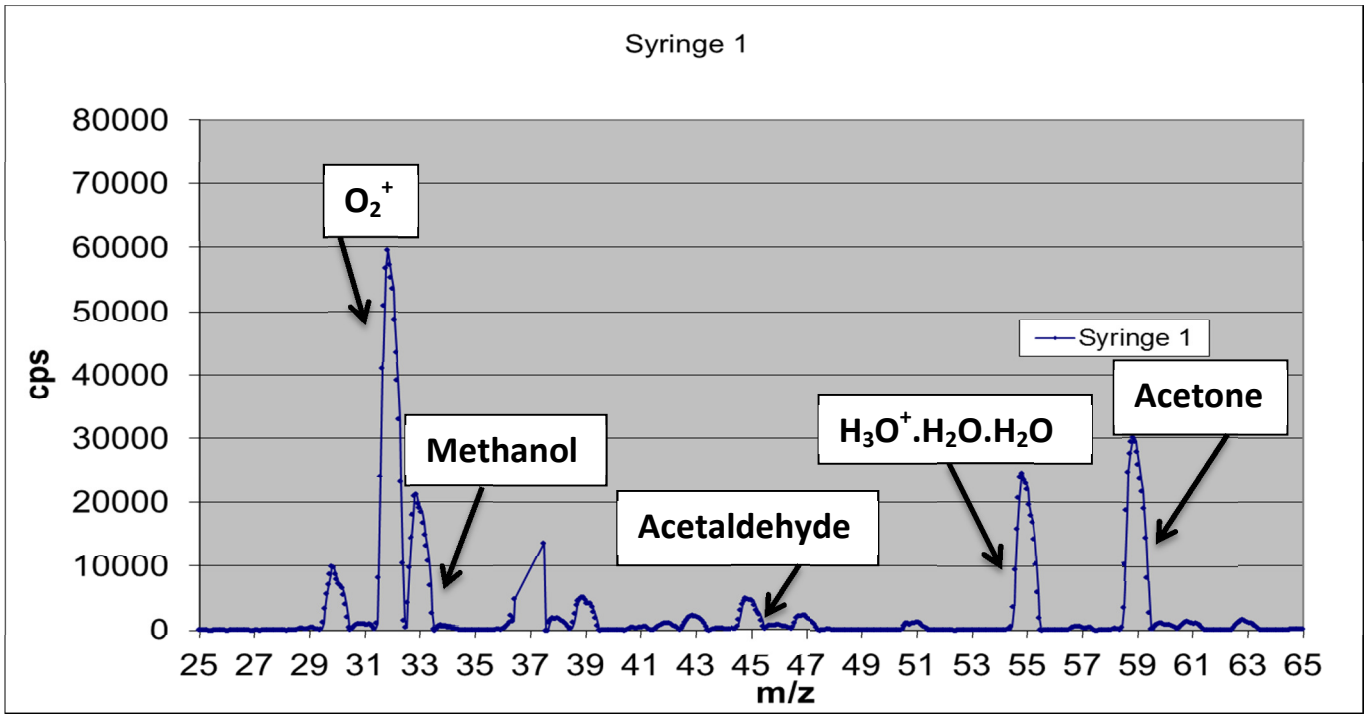


PTR-MS (Proton Transfer Reaction Mass Spectrometer)

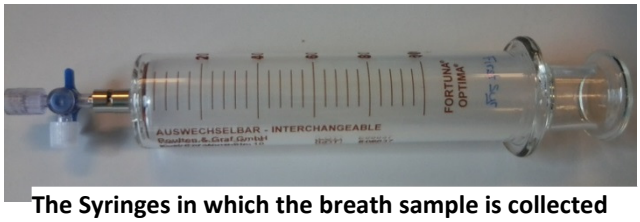
pass through the mass spectrometer allowing the formation of a graph displaying a spectrum (example shown below) of the different VOCs present in the sample.

The following equation shows the reaction which takes place within the mass spectrometer. However it only works if the Proton affinities of VOC are higher than that of the water.

$$H_3O^+ + VOC \rightarrow VOC.H^+ + H_2O$$



As the PTR-MS is really sensitive a lot of care must be taken to ensure that contamination from other sources is minimal. Much of the contamination can occur from the collection equipment and the surrounding air. Previously the breath samples were collected in sample bags however this led to VOC surface adsorption and VOC's were able to diffuse into and out of the bags which resulted in contamination of the sample. This is why glass syringes of 100ml are now used to collect the samples; they reduce the overall contamination of the sample and diffusion of VOC's no longer occurs. However using syringes hasn't completely minimised contamination. Stopcocks made of plastic are



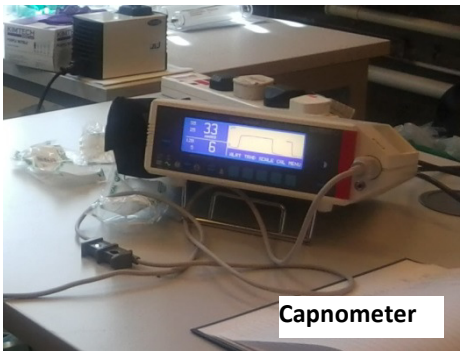
The Syringes in which the breath sample is collected

used to close the syringe ends. These stopcocks are made of plastic which emits VOCs which can contaminate the sample. The stopcocks are baked at 80 °C to decrease the amount of VOCs emitted by them. This is because baking them promotes the release of VOCs leaving less VOC's to be transferred into the syringes.

The Stopcocks being baked in the incubator

Breath Sampling:

A Capnometer measures the concentration of Carbon dioxide in respired gases. The end tidal CO_2 from the alveoli is measured because the alveoli are in contact with the blood and VOCs are measured from the blood. Contamination of the upper airway from the room air is prevented. The capnometer measures the amount of infrared light absorbed by the CO_2 in the gas sample. This is then presented as a capnogram or a capnograph.


Capnometer

Steps:

1. First calibrate the CAPNOGARD (Novamatrix) and place the breathing apparatus into the cell.
2. To clean the syringes to be used, flush them with nitrogen three times. (6 syringes were used)
3. Label the syringes in order to be able to differentiate the different samples.
4. Next measure the concentration of VOCs in the lab air.
5. Allow time for the patient to relax so that their VOC levels don't suddenly rise or decline; then collect the first sample.
6. Attach the first sample immediately to the PTR-MS , measure the spectrum and record the time.
7. Allow the patient to relax and collect a further five samples which will give a total of six samples for the investigation.
8. Place the syringes containing these samples into an incubator at 24°C or into Sample bags.
9. Measure the next syringe containing a breath sample with the PTR-MS 30 minutes after the first syringe.
10. Continue to do so with an interval of 30 minutes (First syringe stored for 30 minutes next for 60 minutes etc.) until all syringes containing breath samples have measured.

This was repeated on another subject on a different day. For both the experiments the dwell time was 0.5s for the spectra. Subject A's breath was collected on the first day and subject B's on the other day. Subject A was a healthy 30 year old female and subject B was a healthy 44 year old female.

Results:

All data has been normalised to 4.5 million CPS (counts per second) of hydronium. The concentration of the proton donor changes from sample to sample so in order to make comparison between two or more data sets easier, the data is normalised. The VOCs are protonated except O_2^+ which is charged by a direct charge transfer.

The Noise Statistic (NS) and Standard Deviation (SD) were calculated using excel. The noise statistic effectively enables the SD associated with any mean signal for any dwell period to be reliably predicted. An example of the formulas used to calculate the SD and NS in excel are shown below.

NS - B170/(SQRT((B170)*0.5))

SD - STDEV(M167:R167)

Tables 1 and 2 show the data for the VOC's: Oxygen, Methanol, Acetic Acid, Acetaldehyde, Etanol, Acetone/Propanal, Propanol, Isoprene, Propanoic Acid.

Subject A Data:					
VOC	**Formula**	**M/Z Amu**	**Peak Heights (CPS) (Noise Statistic)**	**Mean (SD %)**	**Standard Error of the Mean**
Oxygen	O_2^+	32	57900 (340) 64900 (360) 61100 (350) 51200 (320) 52500 (320) 59100 (340)	57800 (11%)	1080
Methanol	CH_3OH	33	9430 (140) 8310 (130) 9090 (140) 10100 (140) 9200 (140) 8700 (130)	9140 (8%)	129
Acetic Acid	CH_3COOH	43	5820 (110) 4720 (97) 5020 (100) 6750 (120) 4900 (99)	5450 (51%)	555
Acetaldehyde	CH_3CHO	45	6420 (110) 7200 (120) 7430 (120) 8660 (130) 8430 (130) 7270 (120)	7570 (14%)	174
Ethanol	C_2H_5OH	47	2430 (69) 2390 (69) 2490 (70) 3930 (88) 3280 (81) 2520 (71)	2840 (28%)	131
Acetone/Propanal	C_2H_6/C_2H_5CHO	59	172000 (590) 149000 (550) 161000 (570) 167000 (580) 166000 (580) 154000 (560)	162000 (7%)	1810
Propanol	CH_3COOH	61	2440 (70) 2130 (65) 1830 (61) 5080 (100) 2360 (69)	4650 (130%)	991
Isoprene	C_5H_8	69	1330 (52) 1200 (49) 1320 (51) 1190 (49) 1020 (45) 826 (41)	1150 (21%)	40
Propanoic Acid	C_2H_5COOH	75	336 (26) 352 (27) 246 (22) 740 (39) 535 (33) 339 (26)	425 (53%)	37.7

Table 1 Showing data for subject A

Due to a software error the data point of Propanol for syringe 4 and Acetic Acid for syringe 4 have been removed from the analysis.

Subject B Data:					
VOC	**Formula**	**M/Z Amu**	**Peak Heights (CPS) (Noise Statistic)**	**Mean (SD %)**	**Standard Error of the Mean**
Oxygen	O_2^+	32	53500 (330) 54600 (330) 58200 (340) 58800 (340) 57100 (340) 58500 (340)	40200 (5%)	429
Methanol	CH_3OH	33	19100 (200) 18300 (190) 16700 (180) 16100 (180) 15500 (180) 14500 (170)	11800 (12%)	330
Acetic Acid	CH_3COOH	43	2170 (66) 2480 (70) 1810 (60) 2040 (64) 1620 (57) 1630 (57)	1390 (20%)	64.8
Acetaldehyde	CH_3CHO	45	4640 (96) 4490 (95) 4520 (95) 4450 (95) 4350 (93) 4130 (91)	3130 (5%)	33.8
Ethanol	C_2H_5OH	47	1930 (62) 2090 (65) 2100 (65) 1940 (62) 1760 (59) 1710 (59)	1360 (10%)	31.1
Acetone/Propanal	C_2H_6/C_2H_5CHO	59	27800 (240) 25300 (230) 23000 (210) 21900 (210) 21400 (210) 22800 (210)	16800 (12%)	470
Propanol	CH_3COOH	61	1070 (46) 1160 (48) 1050 (46) 787 (40) 715 (38) 890 (42)	669 (21%)	33.6
Isoprene	C_5H_8	69	613 (35) 428 (29) 440 (30) 415 (29) 560 (34) 381 (28)	335 (22%)	17.7
Propanoic Acid	C_2H_5COOH	75	175 (18) 207 (20) 200 (20) 113 (15) 74 (12) 70 (12)	99 (51%)	12

Table 2 Showing data for subject B

The concentration of certain VOCs was highlighted to see the differences between the amounts of each VOC present in the breath of both subjects. The uncertainty bars represent the uncertainty in the noise statistic and the pink lines represent the Standard Deviation for all 6 data point. The green lines represent the mean for the data set.

O_2^+ (m/z – 32)

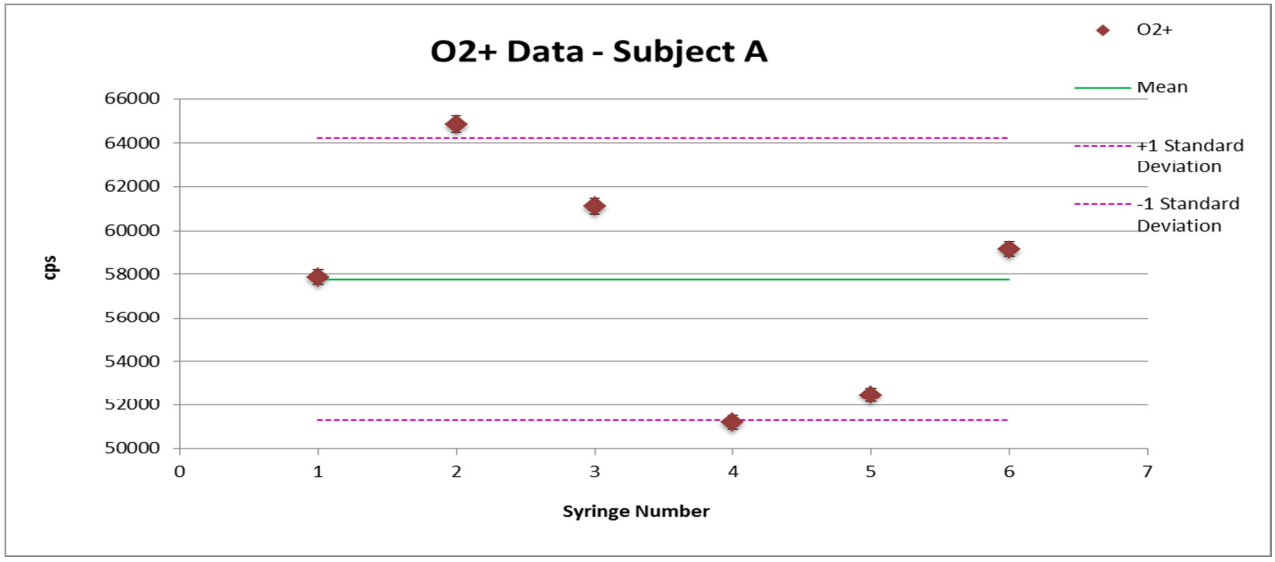


Figure 1: displays the cps against the syringe number and shows the normalised cps for 6 syringes for O_2^+ for Subject A

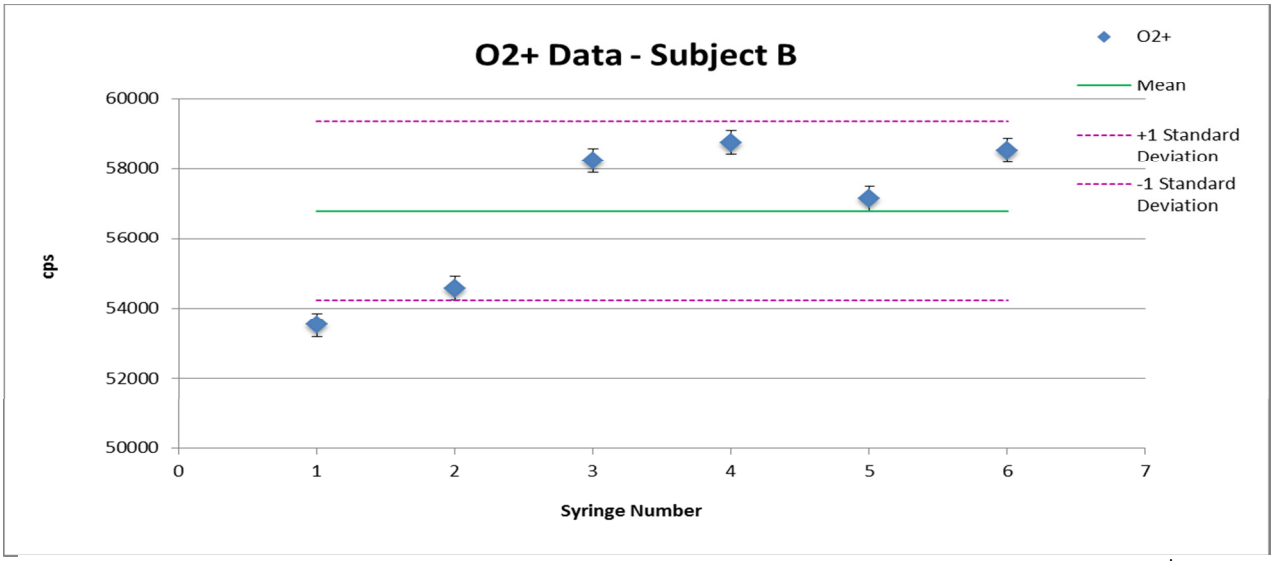


Figure 2: displays the cps against the syringe number and shows the normalised cps for 6 syringes for O_2^+ for subject B

The levels of O_2^+ in Subject A's breath are fluctuating over time whereas subject B's breath shows a gradual increase of O_2^+ levels. The O_2^+ levels are all pretty much within one standard deviation.

Methanol (m/z – 33)

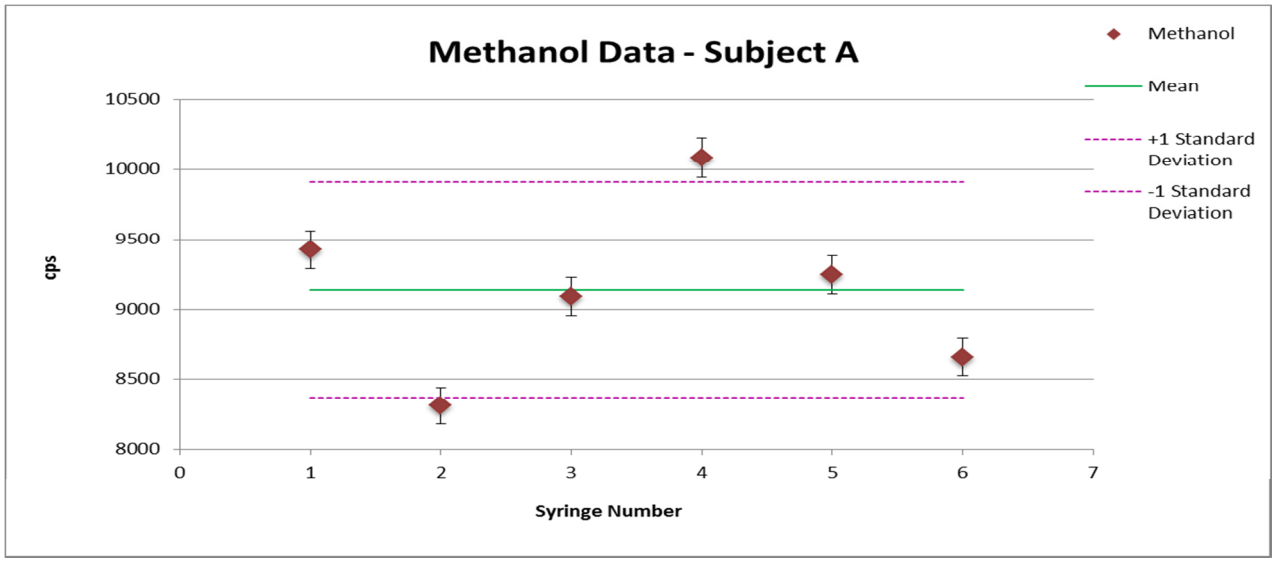


Figure : displays the cps against the syringe number and 3 shows the normalised cps for 6 syringes for Methanol for Subject A

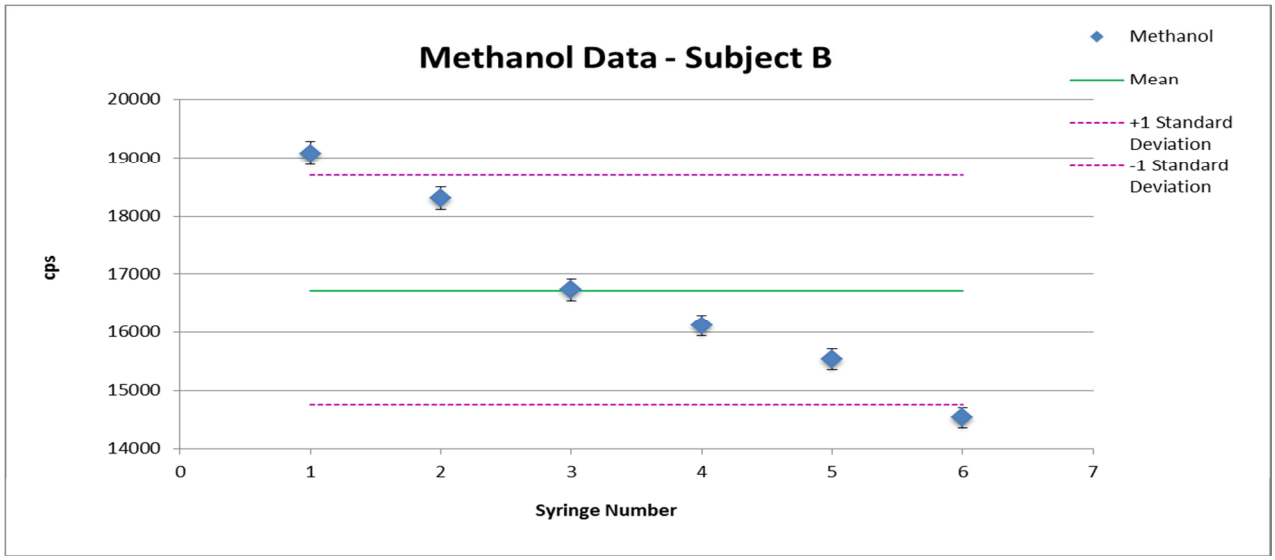


Figure 4: displays the cps against the syringe number and shows the normalised cps for 6 syringes for Methanol for Subject B

The levels of methanol show some fluctuation for subject A but are continually decreasing for subject B. The results for subject B are what would normally be expected in such an investigation. The methanol levels of both subjects within each syringe are within one standard deviation of one another.

Acetic Acid (m/z – 43)

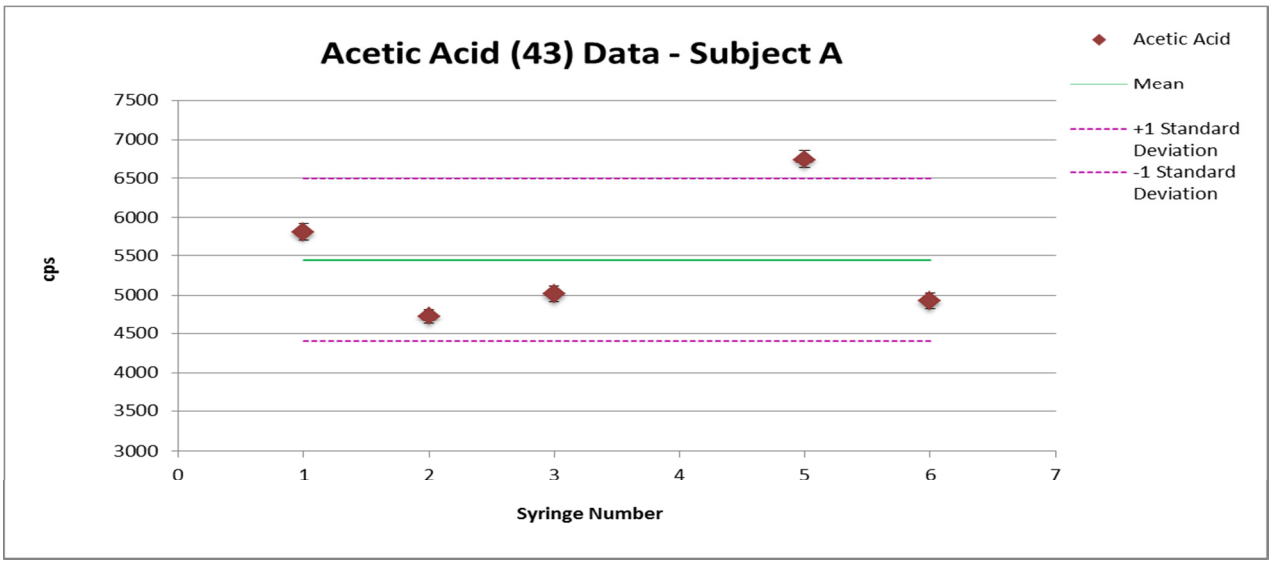


Figure 5: displays the cps against the syringe number and shows the normalised cps for 6 syringes for Acetic Acid for subject A.

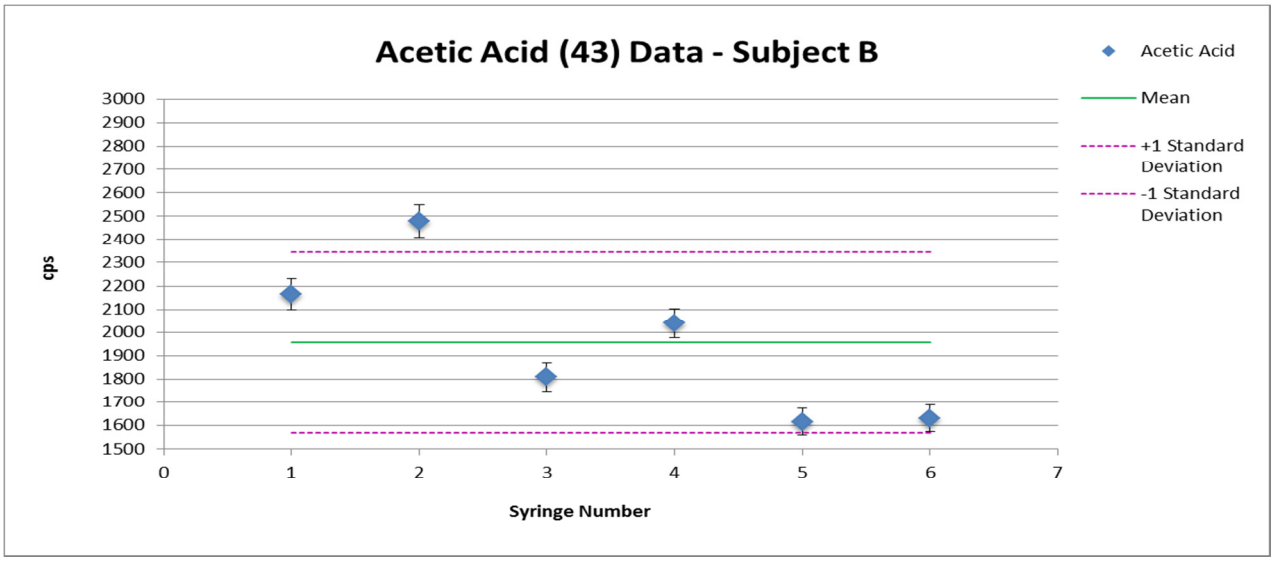


Figure 6: displays the cps against the syringe number and shows the normalised cps for 6 syringes for Acetic Acid for Subject B

The levels of acetic acid in the breath of subject B are considerably lower than in Subject A's breath. Syringe 4 for subject A has incredibly high levels of Acetic Acid and this was due to a software error which is why it was removed from the data analysis.

Acetaldehyde (m/z – 45)

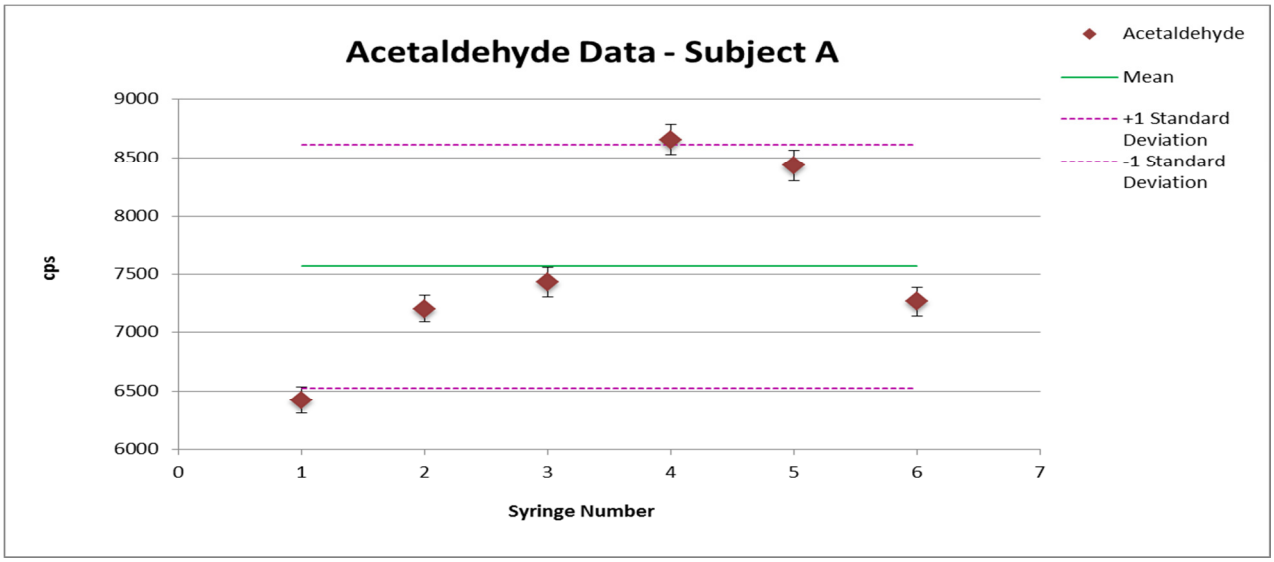


Figure 7: displays the cps against the syringe number and shows the normalised cps for 6 syringes for Acetaldehyde for Subject A

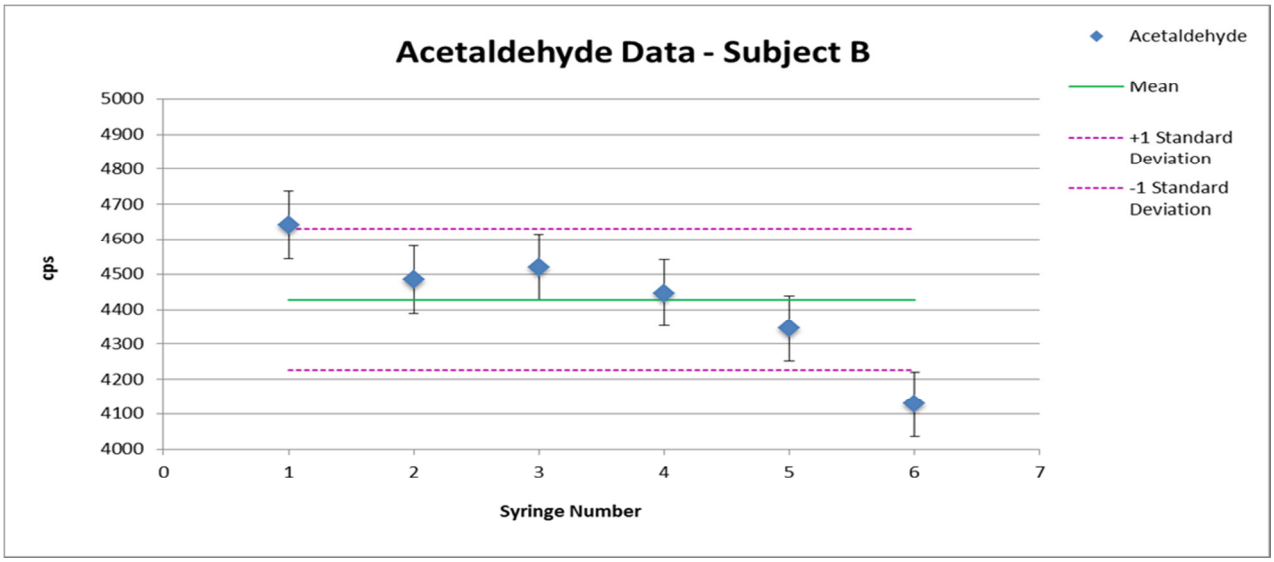


Figure 8: displays the cps against the syringe number and shows the normalised cps for 6 syringes for Acetaldehyde for Subject B

The levels of Acetaldehyde are a lot higher in Subject A's breath than in subject B's. Acetaldehyde is thought to be from ethanol metabolism.

Ethanol (m/z – 47)

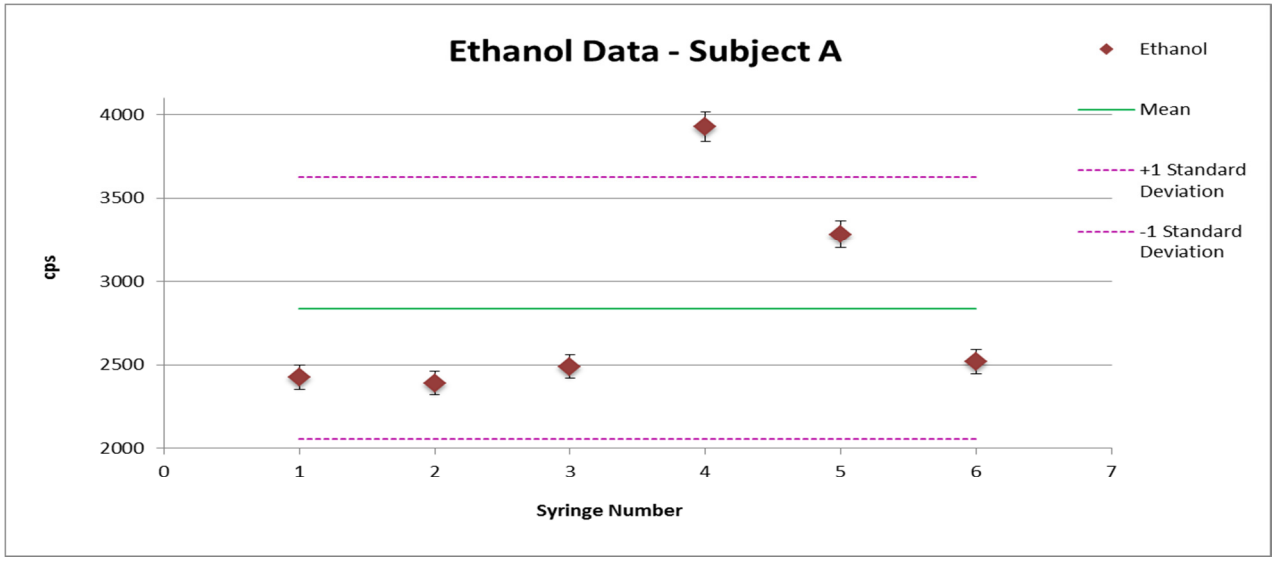


Figure 9: displays the cps against the syringe number and shows the normalised cps for 6 syringes for Ethanol for Subject A

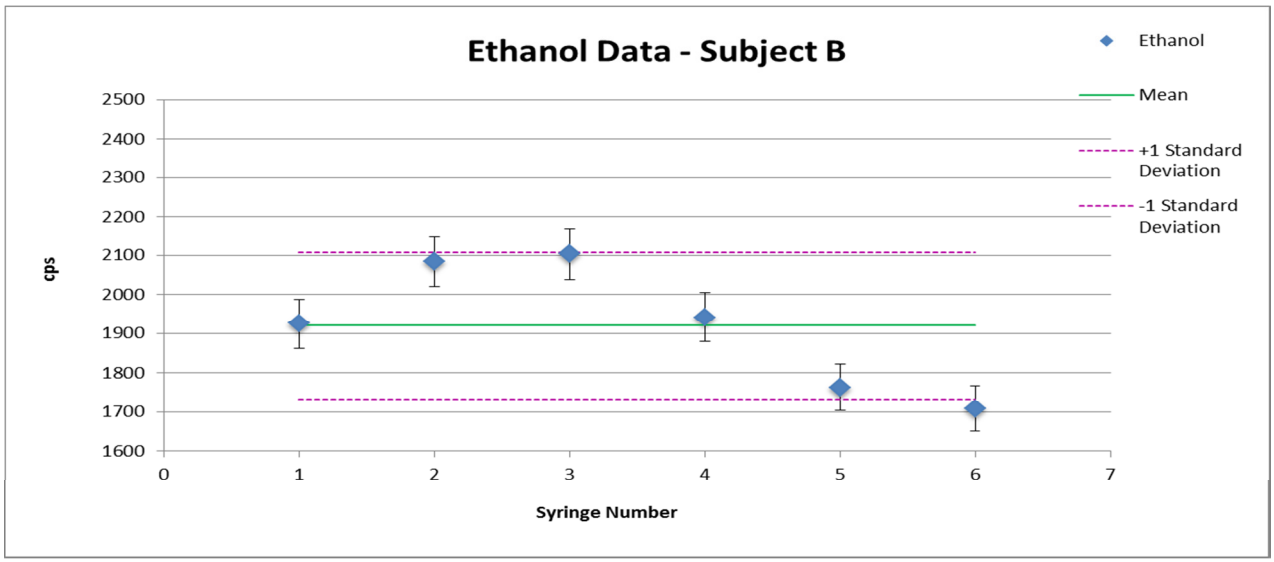


Figure 10: displays the cps against the syringe number and shows the normalised cps for 6 syringes for Ethanol for Subject B

For subject A the levels of Ethanol are very high in syringe 4. Ethanol is present from metabolism of bacteria in the gut this may be why the levels of Ethanol in subject A are very high.

Acetone/Propanal (m/z – 59)

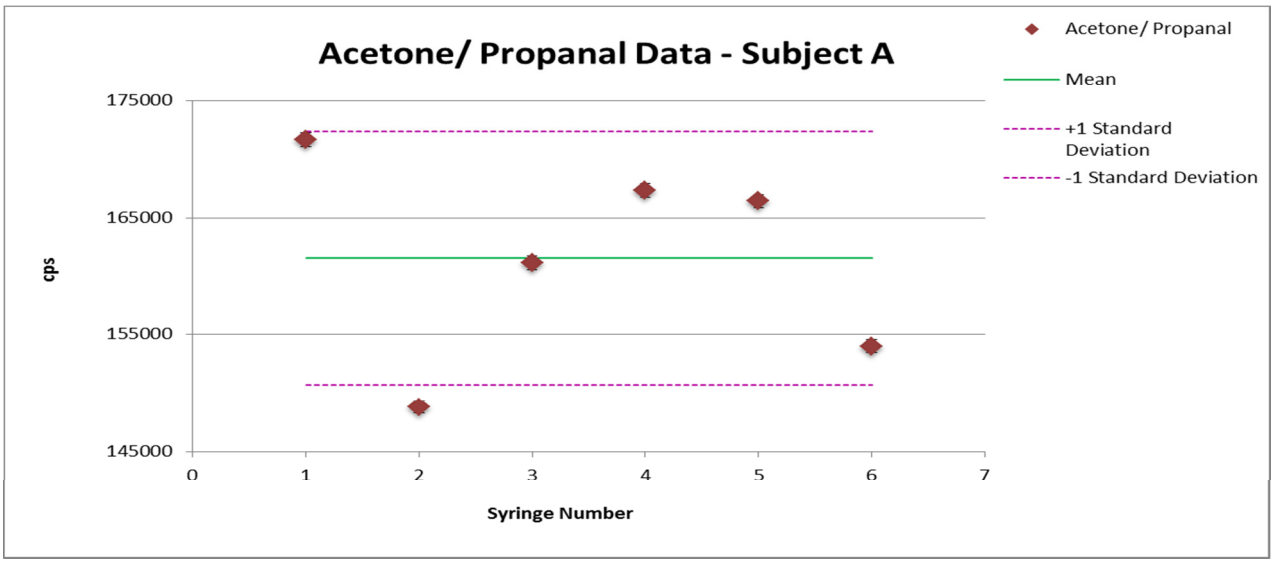


Figure 11: displays the cps against the syringe number and shows the normalised cps for 6 syringes for Acetone/Propanal for Subject A

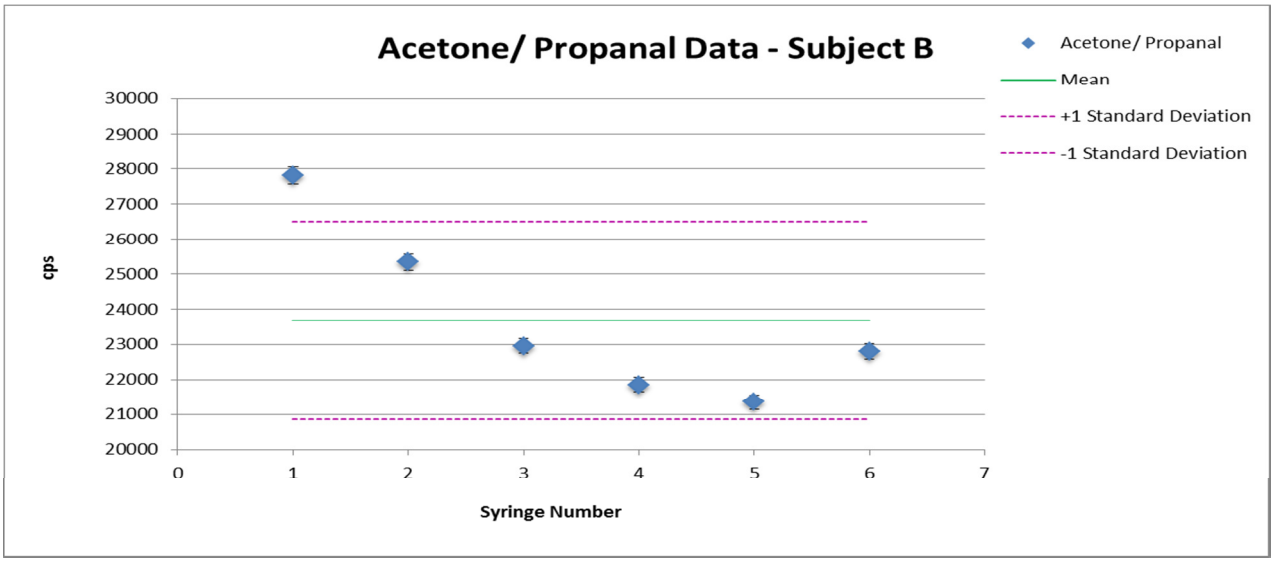


Figure 12: displays the cps against the syringe number and shows the normalised cps for 6 syringes for Acetone/Propanal for Subject B

Subject A's levels of Acetone/Propanal fluctuate a lot more than subject B's. There is little variation in the acetone/propanal levels for subject B which is why the standard deviation is a lot smaller than for subject A. In figure 14 the error bars do not show up as they are smaller than the plotted data point hence not of much significance. Acetone arises from glucose metabolism and subject A had not eaten carbohydrates at breakfast, so perhaps this is why it was so high.

Isoprene (m/z – 69)

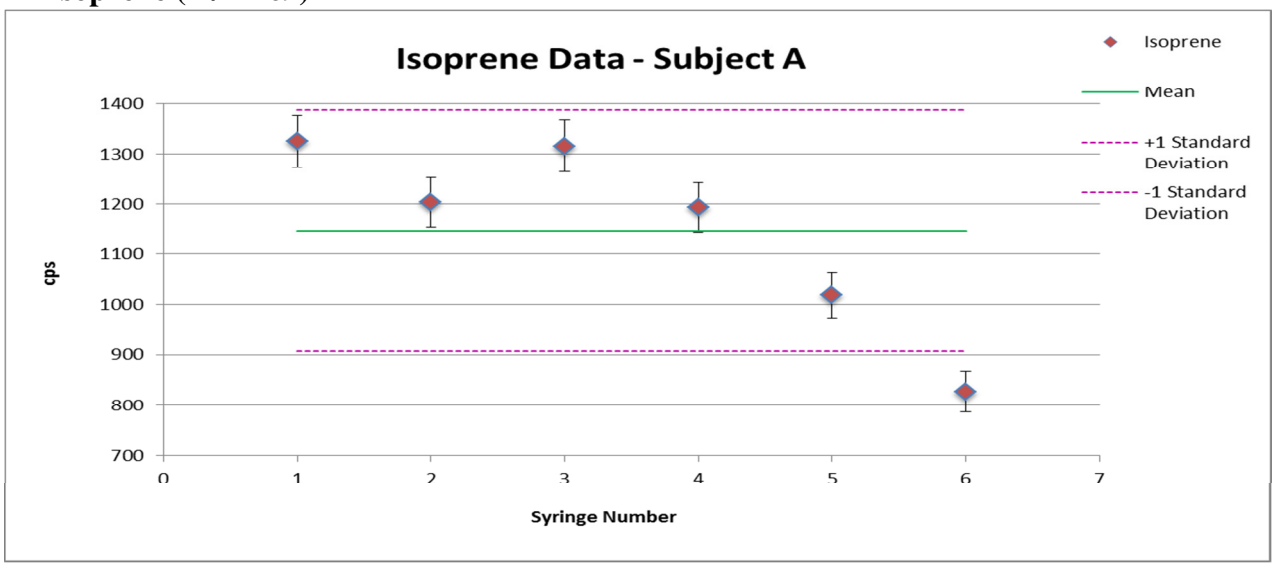


Figure 13: displays the cps against the syringe number and shows the normalised cps for 6 syringes for Isoprene for Subject A

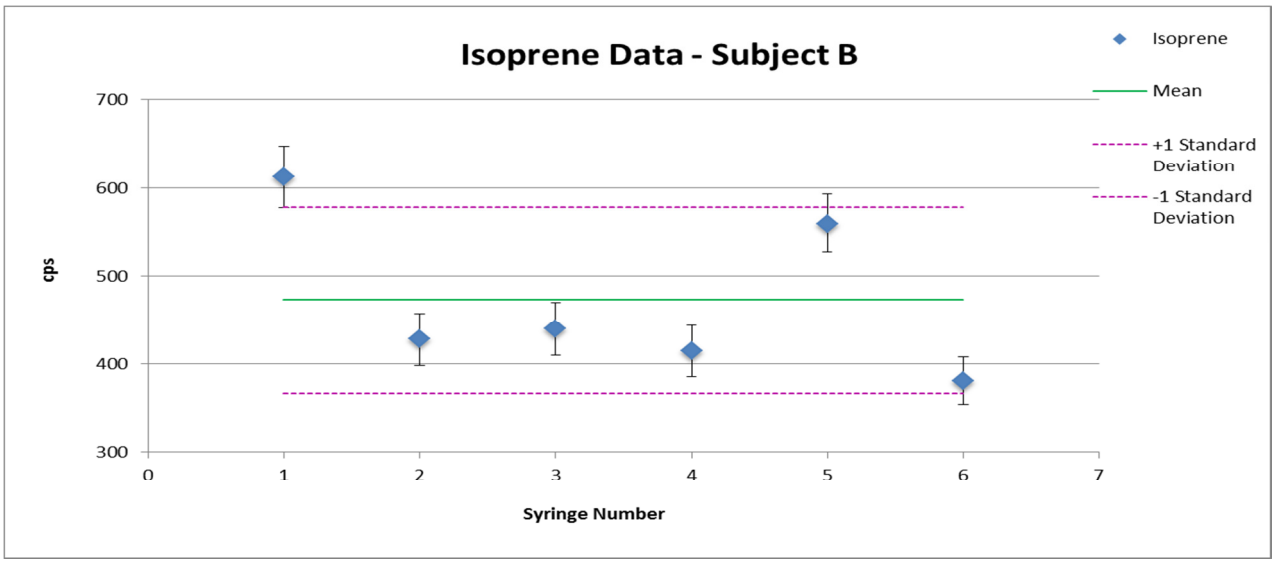


Figure 14: displays the cps against the syringe number and shows the normalised cps for 6 syringes for Isoprene for Subject B

Subjects A's breath samples contain higher levels of isoprene. Isoprene comes from synthesis of cholesterol. It's well known to be very variable in breath because it's affected strongly by heart rate and muscle movement.

Propanoic Acid (m/z – 75)

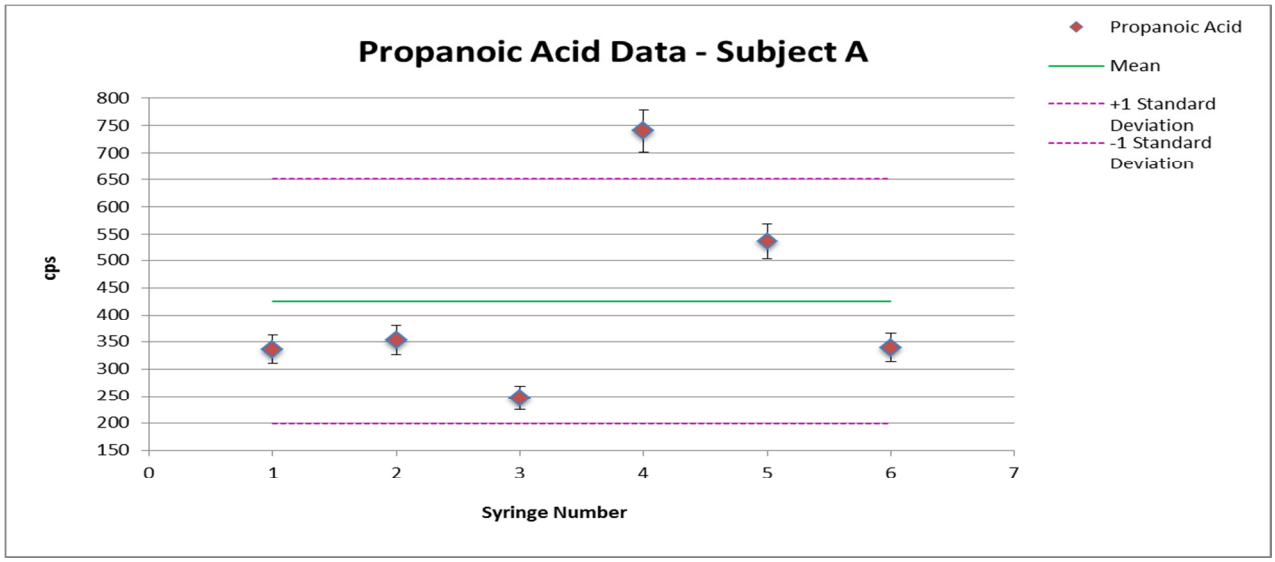


Figure 15: displays the cps against the syringe number and shows the normalised cps for 6 syringes for Propanoic Acid for Subject A

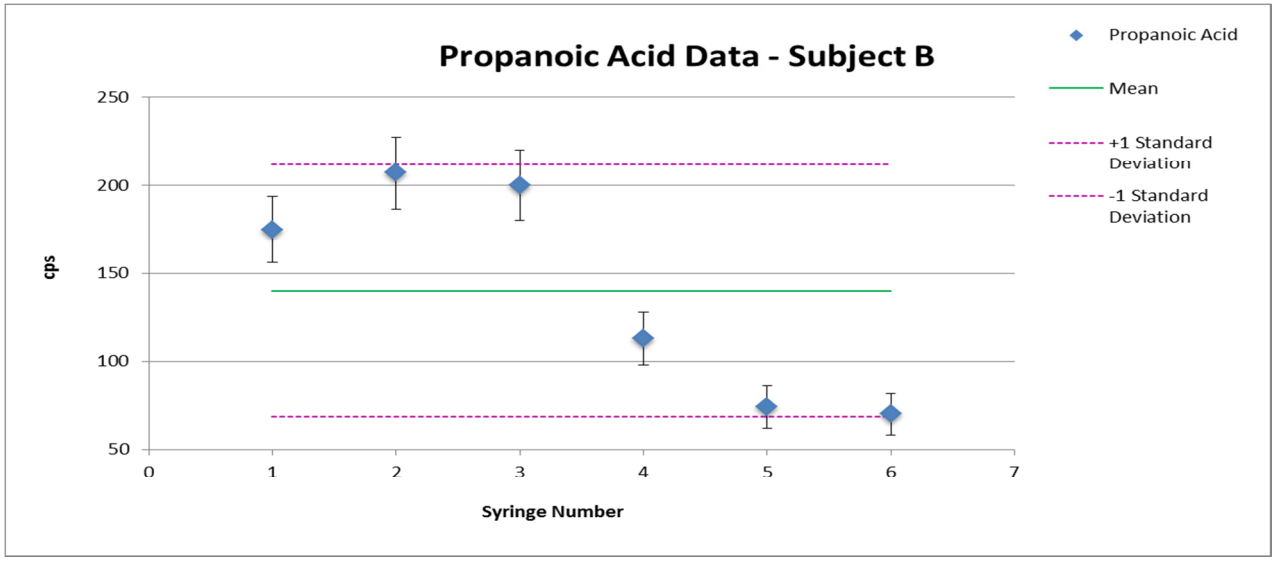


Figure 16: displays the cps against the syringe number and shows the normalised cps for 6 syringes for Propanoic Acid for Subject B

The levels of Propanoic Acid have shown a great decrease for subject B. The levels of Propanoic Acid in syringe 4 for subject A is a lot higher as compared with the other syringes.

Discussion:

Overall Subject B's results for methanol, acetaldehyde and ethanol do show a decrease but the other VOC's don't. However this is not replicated by subject A's data hence we cannot conclude from this that the concentration of VOC's decreases. The test would have to be carried out several more times. This would give more reliable data and a more valid conclusion. Also the trends for each VOC aren't very similar especially for subject A which is why a clear conclusion cannot be reached.

For all VOC's the Standard Deviation of six data points is much larger than the noise statistic of each individual data point. This indicates that variability from breath to breath is much greater than variability's due to counting statistics. This indicates a real difference between breaths even within the same person. This is why a sample of breath even from the same patient can be very different. As a result it may be better to collect one long sample of breath into a bag from each patient. From this sample a certain amount can be put into 6 separate syringes and the rest of the test can be carried out. This may result in less variation within the breath of one subject.

To reach a reliable conclusion the test would have to be carried out on a larger sample and the necessary changes mentioned above would need to be taken. Although the results obtained are promising this investigation has neither proved nor disproved my hypothesis. In order to do this, further studies will need to be carried out.

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