Journal of Chromatography B, 879 (2011) 2815–2820

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Determining the limits and confounders for the 2-pentyl furan breath test by gas chromatography/mass spectrometry

Shrawan Bhandari[a], Stephen Chambers[b], John Pearson[a], Mona Syhre[c], Michael Epton[b], Amy Scott-Thomas[a,*]

[a] University of Otago, Department of Pathology, P.O. Box 4345, Christchurch 8140, New Zealand
[b] Canterbury District Health Board, Private Bag 4710, Christchurch 8140, New Zealand
[c] Leipzig University, Department for Inner Medicine, Neurology and Dermatology, Jahannisallee 32, Leipzig 04103, Germany

ARTICLE INFO

Article history:
Received 13 May 2011
Accepted 10 August 2011
Available online 17 August 2011

Keywords:
Aspergillus fumigatus
Invasive aspergillosis (IA)
Breath test
GC/MS
SPME and 2-pentyl furan (2-PF)

ABSTRACT

Aspergillus fumigatus produces 2-pentyl furan (2-PF), a volatile compound not produced by many other pathogens or normal human metabolism. 2-Pentyl furan has been detected in the breath of patients with invasive aspergillosis (IA) by SPME pre-concentration coupled with CG/MS providing the possibility of an attractive diagnostic test. The limit of detection (LOD) and quantification (LOQ) for peak integration were assessed both statistically and empirically respectively. 2-Pentyl furan was detected from 10 of 45 food stuffs tested. Levels were highest from soymilk (3 of 3 brands), lower from pumpkin, peanuts, rolled oats 2, Ensure Plus®, tinned asparagus, tinned beans and a vegetable exact (Marmite™). No 2-PF was detectable in anti-fungal medications used to treat IA or commonly used cosmetics tested. There was no difference in 2-PF breath levels between morning and afternoon or fasting and non fasting samples taken from healthy subjects eating a diet without 2-PF rich foods. 2-Pentyl furan levels were present in breath samples immediately after a mouth rinse with soy milk ($P < 0.001$), and in some subjects after ingesting soy milk and rinsing their mouth with water. The breath test for 2-PF can be conducted without an overnight fast or at a specified time provided the mouth has been rinsed 30 min or more from when 2-PF containing products have been ingested.

1. Introduction

2-Pentyl furan (2-PF) is a volatile organic compound (molecular weight of 138 g/mol, vapour pressure is estimated to 160 Pa at 25 °C) that is produced by *Aspergillus fumigatus*. This compound may be sufficiently specific to form the basis of a breath test for invasive aspergillosis (IA) as it is not produced by many other respiratory pathogens *in vitro* (except for *Fusarium* spp. and *Streptococcus pneumoniae*) or normal human metabolism [1]. *A. fumigatus* is a ubiquitous mould that causes invasive pulmonary disease in the immuno-compromised population worldwide and has a very high mortality in this patient group [2]. New approaches to diagnosis are needed as current diagnostics techniques such as bronchoscopy and biopsy, PCR of peripheral blood and galactomannan testing have significant limitations both for diagnosis and monitoring of response to treatment [2,3]. A breath test for respiratory infection is an attractive option because of the proximity of the sample to the lesion and ready access to breath samples, that are simple and painless to collect and repeatable even in young children.

Previous studies have shown that 2-PF can be detected in the breath of patients with chronic lung disease colonised with *A. fumigatus*, and has been reported in the breath of immune suppressed subjects with IA [1,2]. However, in patients with chronic lung disease the sensitivity and specificity of the 2-PF breath test were 77% and 78% respectively [2] suggesting that false positive results may reduce the accuracy of the test. Studies are needed to determine the possible source of the false positive results and optimise the performance of this technique.

There are several environmental sources of 2-PF that could confound the results of a breath test. 2-Pentyl furan has been identified as a component of the volatile decomposition products of autoxidised soybean and cottonseed oils [3–5], is sold as a flavour enhancer [6] and has also been reported in a concentration of 0.05–0.1 ppm in roasted coffee beans [7]. 2-Pentyl furan has also been reported in asparagus, rice, potatoes, pork and parsley among other food products [8–14] and as a marker for sick building syndrome [15].

This study set out to optimise performance of the 2-PF breath test by determining the effect of fasting, time of day on baseline results, and to determine if common foods, medication or cosmetics contained 2-PF and whether ingestion of 2-PF rich foods altered breath 2-PF levels in healthy subjects.

* Corresponding author. Tel.: +64 3 378 6209; fax: +64 3 364 0009.
E-mail address: amy.scott-thomas@otago.ac.nz (A. Scott-Thomas).

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doi:10.1016/j.jchromb.2011.08.010

2. Materials and methods

2.1. Materials

2-Pentyl furan, hexamethyldisilazane, 1 L glass sampling bulbs and 2 L Tedlar® bags were purchased from Sigma–Aldrich, St. Louis, Missouri, USA. ChroMAR® methyl alcohol was obtained from Mallinckrodt Baker Inc., USA.

2.2. Solid phase microextraction (SPME)

SPME fibres (StableFlex, DVD/CAR/PDMS) (Supelco, Bellefonte, Pennsylvania, USA) were used to pre-concentrate 2-PF from the headspace of all samples. Each SPME fibre was pre-conditioned in a hot injector port at 250 °C, a test chromatogram was recorded and the clean and activated fibre was then exposed within the sample headspace for the incubation period [1,16].

2.3. Detection of 2-PF by gas chromatography/mass spectrometry (GC/MS)

A Saturn 2200 system (Varian, Palo Alto, United States of America) was used to perform the GC/MS analysis. A Zebron ZB-Wax 30 m × 0.25 mm × 0.25 mm (Phenomenex, Auckland, New Zealand) was coupled to a Programmable Temperature Vaporiser (PTV-1079) injector. The temperatures of the injector, ion trap, manifold and transfer line were 250, 200, 60 and 250 °C, respectively. The oven program commenced at 60 °C for 2 min and was raised to 250 °C at a rate of 10 °C/min, at which the temperature was maintained for a further 2 min. Helium flow was set at a constant rate of 1.2 mL/min. The split vent was opened to a ratio of 1:50 after 1 min. Analysis to detect 2-PF in all samples was performed using the MS–MS capabilities of the ion trap. Ion preparation for MS–MS analysis was EI mode; the selected parent ion was m/z 81 with an isolation window of m/z 3; excitation storage level was 35; excitation amplitude was 35; the resulting MS–MS spectra featured two main peaks at m/z 53 (100%) and m/z 81 (82%).

2.4. Preparation of standard solutions

A stock solution of 2-PF was prepared in analytical grade methyl alcohol which was subsequently diluted to working solutions. The following individual calibration points used were 10.5, 9, 7.5, 6, 4.5, 3, 1.5, 1.13, 0.75, 0.56, 0.23, 0.15 and 0.02 attograms. All 2-PF solutions were stored at 4 °C and were stable for two weeks.

2.5. Calibration

An amount of 10 μL from the working 2-PF solutions ranging from 0.02 to 10.5 attograms were evaporated in Tedlar® bags at room temperature and left to equilibrate for 1 h. These Tedlar® bags contained 2-PF negative breath samples from one healthy subject and the same SPME fibre was used in all of the analysis. Calibration curves were obtained by linear least-squares regression analysis plotting the detector response in terms of peak-area vs. 2-PF concentration.

2.6. Limit of detection and quantification

Two methods were utilised to determine the limit of detection (LOD) and limit of quantification (LOQ). For the statistical method 20 breath samples from one healthy subject were collected over a period of one month. The statistical LOD and LOQ were determined by the following formulae: LOD = mean blank + 3 × standard deviation, LOQ = mean blank + 10 × standard deviation. For the empirical method, the LOD is defined as the lowest analyte concentration that meets predetermined acceptance criteria at least 90% of the time [17]. The criteria were (1) retention time (RT) within ±2% of the 2-PF standard RT. (2) Ratio of ion 53 and ion 81 within ±20%. (3) Base peak (BP) (i.e. relative intensity = 100%) ion 53 or ion 81. (4) Sharp and symmetrical (peak width is consistent with width at high levels) ion peak. (5) Mass spectra are clean with signal to noise ratio of >5. To calculate the precision and accuracy of the method, 11 breath samples from the same healthy individual were collected in 2 L Tedlar® bags over a period of 20 days. These samples were spiked with 10.5 attograms of 2-PF and left to equilibrate for 1 h at room temperature, a SPME fibre was then exposed in the sample for 24 h at room temperature.

2.7. Food and cosmetic analysis

Thirty-five foods, 10 beverages and 10 cosmetic samples were obtained from the local supermarket and/or pharmacy. Any canned good was a product of New Zealand. Replicates of either 200 μL of liquid or 50 mg of each food product were placed into a 2 mL screw top vial with silicone/PTFE screw caps (Chromacol Ltd., Herts, United Kingdom) and allowed to equilibrate for 1 h at room temperature. After 1 h the headspace of each individual sample were analysed and again after heating the sample to 50 °C for 1 min. SPME exposure into the headspace of the vials was at room temperature for 1 min.

2.8. Medication analysis

The four medications tested were obtained from the Christchurch Hospital Pharmacy (Christchurch, New Zealand). Replicates of either 200 μL of liquid medication or 50 mg of powdered medication were placed into a 2 mL screw tip vial with silicone/PTFE screw caps (Chromacol Ltd., Herts, United Kingdom). The medications were left for 1 h at room temperature to equilibrate. The sample headspace was then analysed and once again after heating the sample to 50 °C for 1 min. SPME exposure into the headspace of the vials was at room temperature for 1 min.

2.9. Healthy subjects

Healthy subjects were included only if they were over the age of 18 years, had no known respiratory disease, were not taking prescription or over the counter medication (except oral contraception) and were non-smokers. Breath samples were obtained within 24 h of obtaining consent from each individual.

2.10. Breath sampling

Tedlar® bags were tested for 2-PF before use. All subjects were studied in a facility sourced from a common air conditioning system. No other filters or control systems were used. Breath samples were collected by forced expiration of 1–2 breaths into either a 1 L glass sampling bulb or a 2 L Tedlar® bag, without a nose clip or saliva trap. Breath was collected directly into the bulb or bag without any addition mouthpiece. As soon as the breath was collected the sampling devices were closed ready for analysis. Samples were taken to the GC/MS laboratory within 30 min of collection, preconditioned SPME fibres were inserted via the septa within 1 h. All breath samples contained in sampling devices were incubated with SPME for 24 h at room temperature.

2.11. Fasting vs. non-fasting breath sampling

Ten healthy subjects gave two breath samples over a period of two days into 2 L Tedlar® bags. The first sample was obtained between 7.30 and 9.30 am after 12 h of fasting and the second the

following day, at the same time, after consuming their normal diet. They were asked to avoid any of the following: soy containing products, pumpkin, peanuts, rolled oats, asparagus (tin or fresh), green beans (tin or fresh) and Marmite™ (Sanitarium Healthfood products, Auckland, New Zealand). A food diary was maintained by each subject, including any beverages and or medication taken.

2.12. Diurnal breath sampling

Two breath samples were obtained from 21 healthy subjects in Tedlar® bags. One breath sample in the morning between 7.30 and 9.30 am and another sample in the afternoon between 2.30 and 4.30 pm. No fasting was required by the subjects however they were asked to avoid the foods listed in Section 2.11 and a food and medication diary was again maintained by each subject as in Section 2.11.

2.13. Soy milk mouth rinse without ingestion

Twenty-one subjects fasted overnight for 12 h before giving the first breath sample (7.30–9.30 am). They then rinsed their mouth twice with 30 mL of soy milk (previously tested positive for 2-PF) without swallowing before a second breath sample was collected. Both samples were collected in Tedlar® bags.

2.14. Soy milk ingestion

Ten subjects gave a baseline breath sample before drinking 250 mL of soy milk and then rinsed their mouth with 250 mL of tap water. Further breath samples were taken immediately following the mouth rinse and at 30 min, 1 h, 2 h, 3 h and 6 h post soy ingestion. Subjects were able to eat prior to and during the sampling procedures excluding foods listed in Section 2.11. Food and medication diaries were maintained as in Section 2.11.

2.15. Coffee challenge

Five healthy subjects gave four separate breath samples into glass sampling bulbs. Subjects were asked not to consume any coffee or caffeinated beverages within 12 h of obtaining a time zero breath sample. Subjects consumed a double shot flat white (dairy milk) in 5 min, after which breath samples were obtained at 30 min, 1 h and 2 h.

2.16. Ethical approval

Ethical approval for studies was obtained from the Upper South Island Ethics Committee, New Zealand. All studies were conducted in accordance with the standards for clinical research of the University of Otago, New Zealand.

3. Results and discussion

3.1. Limit of detection and quantification

A linear response was observed from 1.1 to 10.5 attogram of 2-PF spiked in 2 L Tedlar® bags for the ions 53 and 81 (Fig. 1). The R^2 value was reported as 0.98 which was equivalent to that previously published for the 2-PF breath test [1]. Due to this low R^2 value, 2-PF analysis using SPME can only be reported as semi-quantitative at lower concentrations. The mean peak integration and standard deviation for 20 blank breath samples were 732.35 and 526.32, respectively. Thus, using the statistical method, the LOD and LOQ for 2-PF in breath samples were 2311 and 5995 respectively. For the empirical LOD and LOQ, the results obtained from the spiked breath

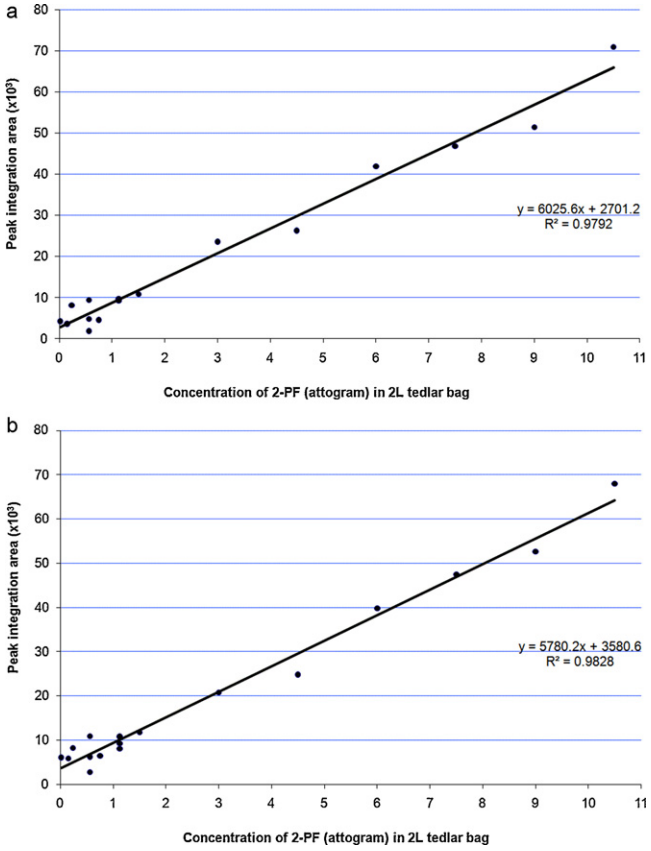

Fig. 1. Calibration curve for the quantification of 2-PF in breath samples collected in 2 L tedlar bags, ions 53 and 81 respectively.

samples were analysed against the pre-determined acceptance criteria. Seventeen of the 20 samples analysed fitted this criteria. Since a concentration of 0.56 attograms of 2-PF did not meet this criteria, 33% of the time, this concentration is the empirical LOD for 2-PF in breath samples. The LOQ for 2-PF was 1.13 attograms. The 2-PF breath test has been shown to have a linear dynamic range out to 50 nmol/mol [1]. The LOD and LOQ for 2-PF in our system varies dependant on which method is used to determine them.

Statistically determined LOD and LOQ values for these types of assay are known to underestimate the true LOD and LOQ. It has previously been reported that the statistical method is not sensitive enough as it measure the average noise level of the procedure and defines only the ability to measure nothing rather than a very low concentration of analyte [18]. Armbruster et al. [17] also reported the statistically determined LOD and LOQ values underestimated the LOD because of the large imprecision associated with blank measurements and the inability of blank samples to meet typical GC–MS acceptance criteria. In their study the empirical method provided much more realistic LOD values [17]. The relative standard deviation (RSD) and error % of the assay was 94.99% and 5.11% respectively.

3.2. In vitro analysis

Fifty-nine samples of foods and commonly prescribed antifungal medications were analysed *in vitro* for the presence of 2-PF in the headspace. Of the 45 foodstuffs analysed, 10 samples tested positive for 2-PF; soymilk (three different brands), peanuts, tinned asparagus (two different brands), one brand of rolled oats, tinned beans, pumpkin and a yeast extract (Marmite™).

2-Pentyl furan had previously been detected in soybean products [3,6,19–21]. 2-Pentyl furan was also detected in Ensure Plus®,

Table 1
Summary of data from breath testing protocols.

Intervention	Subjects *N*	Age years (median)	Female %	2-PF detectable[a] Number (%)		*P* value	2-PF quantifiable number (%)		*P* value
Fasting	10	29–76 (42)	50	Fasting 2(20)	Non fasting 3(30)	NS	Fasting 0(0)	Non fasting 0(0)	NS
Time of day (diurnal)	21	27–76 (38)	57	Morning 7(33)	Afternoon 5(24)	NS	Morning 0(0)	Afternoon 0(0)	NS
Soy mouth rinse	21	27–76 (38)	57	Baseline 7(33)	Post soy rinse 21(100)	<0.001[b]	Baseline 0(0)	Post soy rinse 21(100)	<0.001[b]

[a] Above lower limit of detection by empirical method.
[b] Fisher's exact test.

a soy based nutritional supplement commonly provided to a wide range of patients while hospitalised. A low level of 2-PF was detected in the headspace of the pumpkin sample that had been heated. It is plausible this signal was released from lipid peroxidation of a small part of seed in the sample. Siegmund and Murkovic [22] also described the presence of 2-PF previously in the headspace of pumpkin seed oil. 2-Pentyl furan was also detected in the headspace of peanuts and also in one of the two rolled oat preparations analysed possibly due to a flavour additive. 2-Pentyl furan has previously been detected in both asparagus and beans [8,19] and was once again detected in the headspace of samples tested here. A low level of 2-PF was also discovered in the headspace of a yeast extract sample (Marmite™) both before and after heating. No 2-PF was detected in either instant or freshly ground coffee (4 brands), tea (3 brands), beef mince, pork mince, chicken breast, white fish, cooking oil, olive oil, mushrooms, parsley, peas, potato, cauliflower, cabbage, silverbeet, celery, egg, yoghurt, milk, Fortisip®, walnut, sesame seeds, rolled oats (1 brand), cocoa powder, fresh beans, peanut butter, pizza base, pizza paste, red wine, vinegar and corn tortilla. Other groups have previously reported the release of 2-PF from coffee beans [7] and parsley leaves [12] however we could not verify these results with the experimental set-up described here.

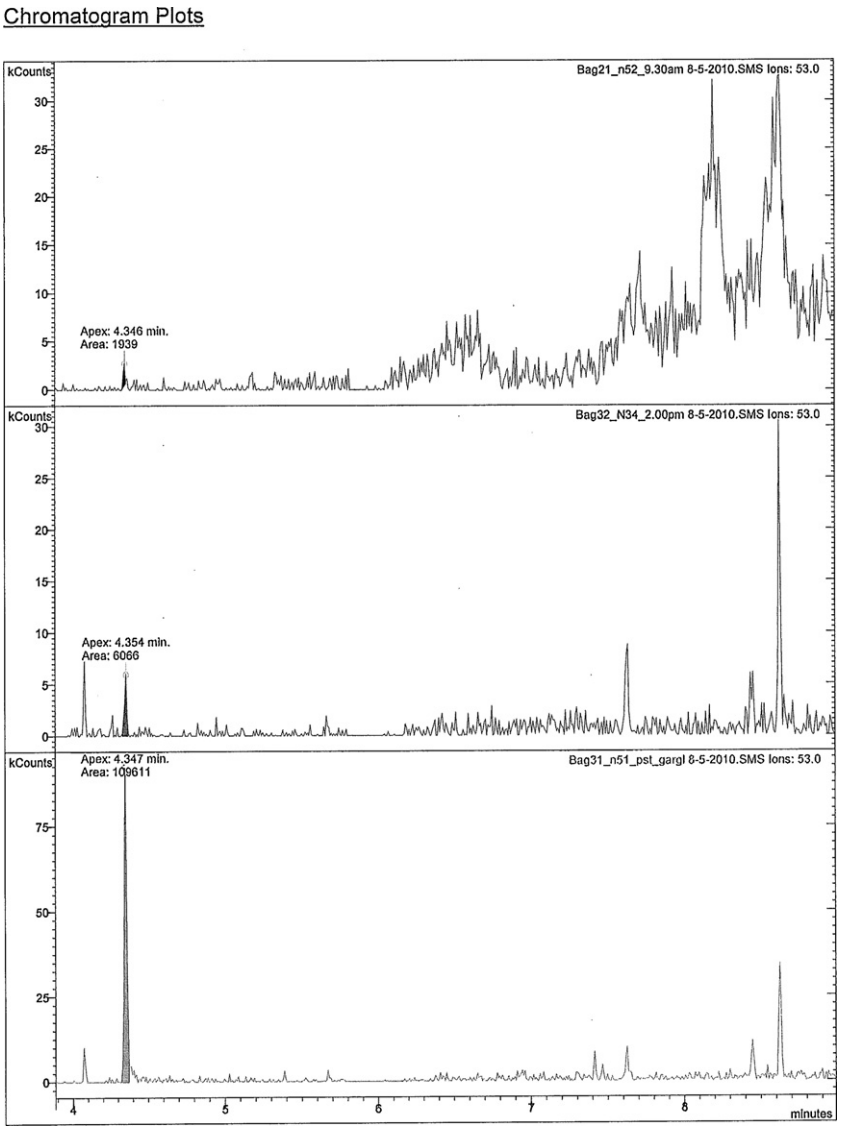

Fig. 2. Comparison of detection of 2-PF in the morning (second panel) and afternoon (third panel) breath samples with that following soy rinse (bottom panel). The top panel shows the mass spectra 53 and 81 in the chromatogram following soy rinse at 4.34 min.

No 2-PF as detectable from 10 cosmetics (2 mouthwash products, toothpaste, after shave, perfume, 2 shampoo products, vaseline, 2 soap products) and four anti-fungal medications (Fluconazole, Itraconazole, Amphotericin B and Voriconazole).

3.3. Fasting and non-fasting breath sampling

Low levels of 2-PF above the lower limit of detection were detected in the breath of 2 fasting and 3 non-fasting subjects but these were below the lower limit of quantification. There were no clear patterns of food ingestion, as documented by a food diary, between those with detectable 2-PF and those without detectable 2-PF in breath. The result indicates that fasting for 12 h does not improve the specificity provided for the 2-PF breath test.

3.4. Diurnal breath sampling

Seven subjects in the morning and five subjects in the afternoon samples had detectable but not quantifiable levels of 2-PF in the breath but only two subjects had detectable levels in both the morning and afternoon. These results show no clear evidence of a diurnal effect in normal volunteers; hence no need to consider time of day when interpreting breath test results.

3.5. Soy milk mouth rinse

A soy milk mouth rinse produced a detectable level of 2-PF in a breath sample in 21 of 21 subjects that was significantly more frequent than in the control samples (7 of 21, $P \leq 0.0001$) and was quantifiable in all samples (mean ± SD 8.5 ± 1.0 attograms) (Table 1). This demonstrates contamination of the mouth is a potential cause of false positive results of a breath test (Fig. 2).

3.6. Soy milk ingestion

As 2-PF could be detected in breath after soy milk mouth rinse, it was necessary to determine if this was removed by a simple mouth wash with water and whether 2-PF may reach the breath after ingestion. Following the water mouth rinse 2-PF was detectable in 6 subjects and quantifiable in 3 (1.1, 1.8 and 1.9 attograms) (Table 2) indicating this procedure was not adequate to remove recently ingested soy product. After 30 min, the levels of 2-PF in breath, and the frequency of detectable but not quantifiable 2-PF signal, were no higher than background. This suggests that a wait of 30 min after soy ingestion prior to breath testing is adequate to eliminate any effects of soy ingestion. It remains possible solid food that is digested and absorbed more slowly could produce a slower release of 2-PF and cause a false positive result but foods had much lower levels of 2-PF making this less likely.

3.7. Coffee challenge

Although no 2-PF was detectable in the four coffee brands when tested *in vitro*, because previous reports confirm the release of 2-PF from coffee [17], it was suggested that 2-PF could be released on the breath after coffee adsorption and metabolism. Of the five subjects tested following coffee ingestion, one had detectable 2-PF in the breath sample but none had detectable or quantifiable levels in subsequent samples.

Table 2
The numbers of subjects with detectable 2-PF in their breath after consumption of soy milk and a water mouth rinse.

Subjects (10)	Time of sampling post water rinse					
	Baseline	5 min	30 min	60 min	180 min	360 min
2-PF detectable	2	6	2	3	1	1
2-PF quantifiable	0	3	0	0	0	0

3.8. Study population normal range

Of the 62 breath samples analysed from normal subjects described above detectable but non-quantifiable levels of 2-PF were found in 47 (76%). The non quantifiable detections were randomly distributed by time and place, consistent with sampling noise. It is possible that low levels of 2-PF are present in the breath of normal subjects or that there is a low level of intermittent environmental 2-PF.

A study population range was therefore determined from data collected from normal subjects in fast/non/fasting and morning/afternoon subjects. As there was no difference in results between these groups by analysis of variance ($P > 0.5$) the data was pooled. The mean count + 3 SD was 1.2 attograms. This was similar to the lower limit of quantification derived from the standard curve and may be a useful cut off for clinical studies using this assay system.

4. Conclusions

Breath analysis offers a non-invasive monitoring option for the fast detection of respiratory diseases such as IA. Obtaining breath is a complex procedure that relies on a pre-defined and rigorous sampling technique. Determining factors that affect the concentration of endogenous VOCs on the breath before it exits the mouth is critical because breath VOCs might originate from either inside or outside the body. We report within, that a number of foods and beverages are indeed potential sources of 2-PF, which is consequently detectable, albeit in very low levels, on the breath of healthy individuals. Knowing the diet of subjects prior to the collection of a breath sample has been shown here to be central in the process of obtaining breath samples and recently ingested 2-PF may give a positive signal. SPME pre-concentration with GC/MS offers a satisfactory method for detection of 2-PF down to levels of about 1 attogram in a breath sample for clinical application, although it may be more sensitive under ideal laboratory conditions. Our results demonstrate that breath tests can be conducted without an overnight fast or at a specified time provided the mouth has been rinsed 30 min or more from when 2-PF containing products have been ingested. Further modification of the current breath collection maneuver may also decrease the problem of false positive results, and improved pre-concentration techniques may improve the accuracy of 2-PF analysis by GC/MS.

Acknowledgements

This work was partially funded by a scholarship from SYFT New Zealand. The authors wish to thank Dr Graeme Wright for all his analytical chemistry expertise.

References

[1] M. Syhre, J. Scotter, S. Chambers, Med. Mycol. 46 (2007) 209.
[2] S. Chambers, M. Syhre, D. Murdoch, F. McCartin, M. Epton, Med. Mycol. 47 (5) (2009) 468.
[3] R. Krishnamurthy, T. Smouse, B. Mookherjee, B. Reddy, S. Chang, J. Food Sci. 32 (4) (2006) 372.
[4] R. Ranau, K. Kleeberg, M. Schlegelmilch, J. Streese, R. Stegman, H. Steinhart, Waste Manag. 25 (2005) 908.
[5] M. Phillips, J. Herrera, S. Krishnan, M. Zain, J. Greenberg, R. Cataneo, J. Chromatogr. B 729 (1999) 75.
[6] T. Smouse, S. Chang, J. Am. Oil Chem. Soc. 44 (1967) 509.
[7] E. Nebesny, G. Budryn, J. JKula, T. Majda, Eur. Food Res. Technol. 225 (2007) 9.
[8] R. Tressl, D. Bahri, M. Holzer, Y. Kossa, J. Agric. Food Chem. 25 (3) (1977) 459.

[9] S. Wongpornchai, K. Dumri, S. Jongkaewattana, B. Siri, Food Chem. 87 (2004) 407.
[10] C. Ho, E. Coleman, J. Food Sci. 45 (4) (2006) 1094.
[11] S. Soncin, L. Chiesa, C. Cantoni, P. Biondi, J. Food Compos. Anal. 20 (2007) 436.
[12] A. MacLeod, C. Snyder, Subramanian, Phytochemistry 24 (11) (1985) 2623.
[13] C. Ho, K. Lee, Q. Jin, J. Agric. Food Chem. 31 (1983) 669.
[14] I. Chung, M. Yeo, S. Kim, H. Moon, Int. J. Food Sci. Nutr. 62 (3) (2011) 215–218.
[15] P. Gao, F. Korley, J. Martin, B. Chen, Am. Ind. Hyg. Assoc. 63 (2002) 135.
[16] S.T. Chambers, S. Bhandari, A. Scott-Thomas, M. Syhre, Med. Mycol. 49 (2011) S54.
[17] D. Armbruster, M. Tillman, L. Hubbs, Clin. Chem. 40 (7) (1994) 1233.
[18] S. Needleman, R. Romber, J. Anal. Toxicol. 14 (1) (1990) 34.
[19] R. del Rosario, B. de Lumen, T. Habu, R. Flath, T. Mon, R. Teranishi, J. Agric. Food Chem. 32 (1984) 1011.
[20] M. Solina, P. Baumgartner, R. Johnson, F. Whitfield, Food Chem. 90 (2005) 861.
[21] S. Yuan, S. Chang, J. Agric. Food Chem. 55 (2) (2007) 426.
[22] B. Seigmund, M. Murkovic, Food Chem. 84 (2004) 367.