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Assessment of Matrix Effects on Methyl Benzoate, a Potential Biomarker for Detection of Outgassed Semi-Volatiles from Mold in Indoor Building Materials

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Abstract Methyl benzoate – as a biomarker for mold growth – was used as a specific target compound to indicate outgassed MVOC products from mold. Both real and surrogate samples were analyzed from a variety of matrices including: carpet, ceiling tiles, dried paint surfaces, wallboard and wallboard paper. Sampling parameters, including: desorption, extraction time, incubation temperature, pH, salt effects and spinning rate, were optimized. Results suggest that extraction and detection of methyl benzoate amongst other MVOCs can be accomplished cleanly by SPME-GC/MS methods. With detection limits (LOD = 1.5 ppb) and linearity (0.999) over a range of 100 ppm to 2 ppb, this work demonstrates that such a green technique can be contemplated for use in quick assessment or as part of an ongoing assessment strategy to detect mold growth in common indoor buildings and materials for both qualitative and quantitative determinations. Of importance, no matrix effects are observed under optimized extraction conditions.

Keywords Biomarkers · Matrix effects · Methyl benzoate · Indoor molds

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A number of volatile organic compounds (VOCs) have been identified as outgassed products from bacteria and mold (Wolkoff et al. 2006; Kuske et al. 2006). Such products have been investigated in hopes that their output concentrations could be linked to growth and to specific species (Reeslev et al. 2003), and thus might be utilized as potential biomarkers for mold detection and assessment. In particular, in enclosed environments, where larger portions of the human population spend more and more time, such elevations in microbial VOCs (MVOC) concentrations have been considered problematic, as they are thought to be linked or associated with poor air quality (Fischer and Dott 2003). Although a clear correlation between MVOC outgassing concentration and poor health has not been demonstrated, there is a growing body of evidence that such correlations might occur (Fischer and Dott 2003; Cooley et al. 1998) especially over prolonged exposure and may in part be related to so-called sick building syndrome. It has been shown that the outgassed volatile and semi-volatile compounds from molds growing in many different types of building materials, can permeate indoor air and subsequently be lysed through the lungs (Miller 1994). Concentrations of MVOC at levels of about 50 ng/dm^3 or greater are thought to be detrimental to health (Sunesson et al. 1995), especially to the more susceptible portion of the population (young, elderly and infirmed).

There are many known methods to assess mold and bacterial growth (for example: culture growth studies, ergosterol analysis, and total fatty acid analysis) however these analytical methods are very time consuming, labor intensive, costly and hence do not lend themselves easily to preventative or to quick detection schemes. As such, a convenient analytical method to quantify such volatiles has not been fully addressed. Previously, we (Wady et al. 2005) and others (Zygmunt et al. 2001) have demonstrated

a preparation and extraction method, using solid phase microextraction (SPME) followed by a GC/MS analysis method to assess such components. Further, a semi-volatile, methyl benzoate, has shown promise as a possible biomarker for the detection of a variety of molds and indications suggest that some correlation to mold growth (Wady and Larsson 2005; Wady et al. 2005).

The difficulty of using VOCs as biomarkers is that their quantitative detection is often susceptible and dependent upon matrix composition (Balakrishnan et al. 2006); since buildings provide and myriad of potential growth surfaces this is problematic. This recent work examines the effects of different matrices upon quantitation, linearity and detection limits for such MVOCs by using methyl benzoate as a representative target analyte. This paper further examines the usage of this green technique, solid phase microextraction coupled to gas chromatography with mass spectroscopy detection (SPME-GC/MS), in application towards to real environmental samples and its ability to act as a rapid analytical method for quick and reliable detection to MVOCs.

Materials and Methods

Methyl benzoate (>99%) was purchased from Sigma-Aldrich (Mississauga, ON, Canada). Ultra-pure water from a Barnstead/Thermodyne NANO-pure water system (Dubuque, IA) was used throughout the study. All solvents used were of HPLC grade. For sampling environmental samples, molds were extracted with a buffered solution (pH 7.0 ± 0.1) containing (g/L): KH_2PO_4 (0.0425), $MgSO_4$ (0.25), NaOH (0.008), and Tween 80 (0.2 mL/L). Helium (99.99%) obtained from Air Liquide (Corner Brook, NL, Canada) was used as a carrier gas for the GC/MS systems. SPME fibers, 100 μm-polydimethylsiloxane (PDMS) and 70 μm-Carbowax/DVB-stableflex (Supelco, Sigma–Aldrich, PA, USA) were conditioned under helium using manufacturers conditions and were reconditioned under helium at 220°C for 2 min between subsequent extractions.

A Varian Star 3400 CX gas chromatograph (Mississauga, ON, Canada) equipped with a splitless/split injector and a Saturn 3 ion trap MS detector was used for all analyses. For manual extractions the sample vial was wrapped with a voltage controlled thermal tape (Omega, Sherbrooke Canada) where the thermocouple wire connected to a rheostat controller was used to maintain and the temperature setting. All timings (extraction, injection, desorption) were monitored by a stop watch, and the stirring was controlled by a PMC Dataplate programmable stirrer (Fisher Scientific, Mississauga, ON). The GC was equipped with a SPME glass insert (Varian) and fused capillary 5%-diphenyl – 95%-dimethyl polysiloxane DB-5MS column (J & W): 30 m × 0.25 mm ID with a 0.25 μm phase (Restek, Brockville, ON). Experimental GC column temperature program conditions were optimized: 90°C for 1 min, heated to 120°C at a rate of 5°C/min and held for 2 min, for a total time of 8 min, using helium as a carrier gas with flow rate of 1.6 mL/min. Samples were injected with closed split at 5 psi. The GC/MS injector temperature was held constant at 230°C and the temperature of both transfer line and ion trap was held at 260°C and 220°C, respectively. The electron multiplier was operating at 2,000 V over the ion trap detection (ITD) mass range of 50–550 m/z.

For all analyses, a headspace extraction method was employed and carried out on 25 mL sample volumes in 30 mL vials which were sealed with screw-top Teflon–Teflon coated septum sealed caps. For quantitation and calibration, an appropriate amount of methyl benzoate was added to 50:50 methanol:water (v/v), to make a stock solution of 50 parts per million (ppm, v/v) and used to prepare, by serial dilution, a series of standards (6, 4, 2, 1, 0.6, 0.2 ppm and 100, 50, 25, 15, 10, 6, 4, 2, 1 and 0.5 ppb) so that each sample had a final volume of 25 mL. Other series of the same standards were prepared for different matrix compositions, which included additions of 250 mg portions of: ceiling tile, dried paint, wallboard, wallboard paper, and solvent extractions from carpet, all in buffered solutions. The solid materials (which contained no glue) were collected from a laboratory prep room at the University were no mold was present and were allowed to stir in the matrix solution for one day to ensure homogeneity before addition of the methyl benzoate standard. The linear range and detection limits were determined from these samples. Further a set of the non-molded samples were prepared under similar conditions to provide sample matrix blanks.

Under manual operation, penetration of the sample by the SPME syringe, exposure of the fiber to the headspace of the vial, and directly followed by desorption at the GC injector was carried out. To ensure that there was no impurity or analyte carry over between samplings, the SPME fiber was reconditioned for 2 min at 220°C before the first run and between runs.

A spiked methyl benzoate (2 ppm) in methanol:water (10:90) sample was used to optimize the experimental SPME extraction and desorption conditions, and included the following parameters: extraction time was varied at 1, 2, 5, 7, 10, 15 and 20 min intervals, spinning time was investigated at 500, 750, 900 and 1200 rpm, extraction temperature was investigated at 20, 25, 30, 40, 60 and 70°C, desorption times of 1, 1.5, 2, 2.5 and 3 min, the effect on extraction by addition of salt comparing 0%, 3%, 10% and 30% concentrations of sodium chloride, sodium sulfate and potassium chloride, and pH conditions were

optimized making up 2 ppm samples in 0.2 M buffers solutions to a series of replicate samples to give samples at pH 2, 4, 5, 5.5, 6, 7, 8 and 9.

Surrogate building material samples of dried paint, ceiling tile, wallboard and wallboard paper (without glue) and carpet samples with visible fungal growth were collected from the same University test room. The samples were cut into small pieces (approximately 3 × 3 mm, 500 mg) and transferred to 30 mL vials containing 25 mL water. For carpet samples, a small portion of the carpet was extracted with a 30% buffer: water solution. Analysis was carried out using the optimum conditions achieved for the fiber.

Results and Discussion

Methyl benzoate, chosen as the target analyte, was based on a previous study (Wady et al. 2005) where it was found to be typical of many outgassed MVOCs from mold. A 100 μm-PDMS and a 70 μm-Carbowax/DVB SPME fiber were tested for performance towards extraction of methyl benzoate. Results indicate that the 70 μm-Carbowax/DVB SPME fiber preformed best and the optimized conditions used for this fiber are given in Table 1. The relative error (%RSD = 3.6) was computed by comparing the total ion chromatograph (TIC) relative integrated peak areas deviations for replicate samples. The value was considered to acceptable for analyses carried out under manual extraction and injections, and could likely be improved if the procedure were to be automated.

Table 1 The optimum extraction conditions achieved for extraction of 2 ppm methyl benzoate from methanol:water (80:20, v/v) solution[a]

Parameters optimized	Parameter intervals tested	Optimum conditions
Extraction time (min)	1, 2, 5, 7, 10, 15, 20	10 min
Spinning rate (10 min)	500, 750, 900, 1200	750 rpm
Extraction temp. (10 min)	20, 25, 30, 40, 60, 70	25°C
Desorption time	1, 1.5, 2, 2.5, 3	2 min
pH (10 min)	1, 4, 5, 5.5, 6, 7, 8, 9	pH 5.5
Salt effect (10 min) (NaCl, Na_2SO_4, KCl)	0%, 3%, 10%, 30%	3% KCl

[a] RSD = ±3.6% relative error, to give 67% extracted with optimum conditions at 10 min. RSD = ±1.7% relative error to give 83% extracted at 25 min using a 70 μm-Carbowax/DVB SPME fiber

Unlike total headspace analysis, SPME is not an exhaustive technique and hence the amount of analyte extracted is highly dependent upon exposure of the fiber to the sample (extraction time). To attempt to achieve the quickest analysis time, a 10 min extraction time was chosen in preference to a 25 min extraction (equilibrium condition), even though greater percentage extracted could be achieved at the longer time (see Table 1). As such the total analysis time (for a 10-min SPME extraction, desorption and GC/MS analysis) was 18 min.

Under the optimized extraction and analytical conditions, calibration curves, linearity and detection limits achieved for methyl benzoate from different sample matrix compositions were determined (Table 2). Methyl benzoate samples greater than 100 ppm gave problems with solubility. GC/MS analysis of blank samples from each of the different sample matrices showed no observable methyl benzoate analyte.

Figure 1 shows an expanded graph of calibration lines for four different matrices. Only small deviations (<1% relative integrated area) occur between all the matrices tested for concentrations below 1.5 ppm (μg/mL). At these levels most environmental analyte concentrations are expected to be found. At higher concentrations, deviations of only about ±3.4% error at 4 ppm, and about ±8.8% error at 5.6 ppm occur. Further, the limits of detection and linearity achieved indicate that the technique response is low and linear to easily assess both standards and detect molds from real samples. Table 3 gives some typical recovery values obtained for samples collected from the laboratory prep room in Environmental Science prep room at the University. For non-moldy samples, samples were spiked with 200 ppb methyl benzoate. The methyl

Table 2 Comparison of matrix effects, linearity and detection limits for 10 min methyl benzoate extractions

Matrix	Calibration curve equation	R^2	Linearity	Detection limit (ppb, ng/mL)
Buffer	y = 11623x + 2250	0.9998	0.002–100 ppm	1.0 [1.2]
Carpet extraction	y = 2404x + 9148	0.9994	0.002–100 ppm	1.5 [1.3]
Ceiling tile	y = 2053x + 2708	0.9997	0.002–100 ppm	2.0 [2.1]
Wallboard	y = 1971x − 5676	0.9997	0.002–100 ppm	2.1 [1.6]
Wallboard paper	y = 2414x + 9174	0.9993	0.002–100 ppm	1.5 [1.4]
Methanol	y = 2414x + 9187	0.9993	0.002–100 ppm	1.2 [1.4]
Methanol:H_2O (80:20)	y = 19713x − 6033	0.9997	to 100 ppm	1.0 [1.1]
Methanol:H_2O (10:90)	y = 19713x − 6033	0.9997	to 100 ppm	1.0 [1.7]

N = 3, [ ] = %RSD

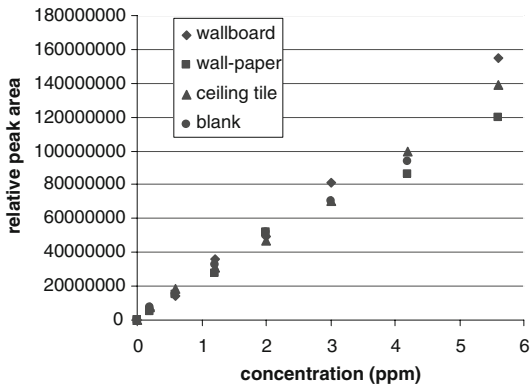

Fig. 1 Calibration curves (0.002–6 ppm) of methyl benzoate for various matrices

Table 3 Detection of methyl benzoate from spiked and non-spiked 500 mg samples

	Detected amount (ppb, ng/mL)
Spiked samples (200 ppb methyl benzoate)	
Carpet extraction buffer	200 [1.9]
Ceiling tile	197 [2.2]
Wallboard	198 [1.7]
Wallboard paper	198 [1.4]
Blank reference methanol:water (10:90)	200 [1.3]
Methanol:H_2O (80:20)	200 [1.4]
Non-spiked samples	
Moldy carpet	15 [2.1]
Moldy ceiling tile	9 [1.5]
Moldy paint	16 [1.8]
Moldy wallboard	11 [1.1]
Moldy wallboard paper	11 [1.1]

N = 3, [ ] = %RSD

benzoate spiked in methanol: water (10:90) matrix was used as a comparative standard. In addition, samples that contained mold were collected from the same room and were subsequently analyzed by the analytical method. Conservatively we conclude that an R^2 value of 0.999 or better can be obtained by this method for any of the matrix conditions studied under optimum extraction conditions. This further gives evidence of reproducibility of the method, and with short analysis times (18 min total analysis), the possibility of this technique to be incorporated into a preventative early detection scheme.

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