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Optimisation of Headspace Solid Phase Microextraction for Gas Chromatography Time-of-Flight Mass Spectrometry Analysis of Pit Latrine Key Odorants

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Solid phase microextraction (SPME) fibre type and main experimental factors and their interactions for optimisation of a headspace solid phase microextraction-gas chromatography time-of-flight mass spectrometry (HS-SPME/GC-ToFMS) for the determination of four key odorants; butyric acid, dimethyl trisulphide, p-cresol and indole, in pit latrine faecal sludge were identified. Carboxen-polydimethylsiloxane (CAR/PDMS) was the SPME fibre that gave best extraction efficiency for all the odorants of interest in this study. All the factors and their interactions were statistically insignificant at α = 0.05 on the total peak areas of DMTS. The main effects; extraction temperature, extraction time and ionic strength were statistically significant at α = 0.05 on the total peak areas of butyric acid, p-cresol and indole. The two-way interactions of extraction temperature and extraction time, extraction temperature and ionic strength, and extraction time and ionic strength were also statistically significant at α = 0.05 on the total peak areas of butyric acid, p-cresol and indole. The sample pH and the interactions of sample pH and ionic strength were statistically significant at α = 0.05 for indole only.

1. Introduction

Pit latrines are still dominant basic minimum acceptable forms of sanitation in low income communities of developing countries (Thye et al., 2011). Disagreeable smells-malodours released from faecal sludge in the pit latrines, which elicit disgusting or repulsive response, are one of the factors that thwart adults and children to use latrines and encourage open defecation as an alternative. This provides an important but often overlooked major impediment, dissuading people from adopting and using the pit latrines hence affecting successful effective sanitation promotion (Rheinländer et al., 2013).

Recent study (Chappuis et al., 2016) revealed that the malodours are attributed to four odorants: butyric acid ($C_4H_8O_2$), DMTS ($C_2H_6S_3$), indole (C_8H_7N) and p-cresol (C_7H_8O). Although these odorants were found in significant concentrations in the headspace of latrines in the developing countries of India and Africa (Chappuis et al., 2015) their human odour threshold are sometimes found in trace and ultra-trace concentrations to be identified and quantified. For this reason, the development of an accurate and reliable analytical method which pre-concentrates the analytes prior to chromatographic analysis is absolutely crucial.

SPME is a popular selective solvent-free sample pre-concentration technique (Benhabib and Town, 2012). The technique is simple, sensitive, reliable, inexpensive, time efficient and easy-to-automate for the analysis of volatile compounds (Souza-Silva, 2013). The SPME sample pre-concentration technique has overcome the limitations of other well established and widely used traditional sample preparation techniques such as solid phase extraction (SPE), purge and trap, liquid extraction and liquid-liquid extraction (LLE) in terms of procedure, accuracy, sensitivity, repeatability, simplicity, cost and greenness (Piri-Mughadam et al., 2016).

However, the different possible configuration of SPME and sample preparation steps directly affects the results of the analysis. The SPME method optimisation has been achieved by a traditional univariate procedure, in which one experimental factor is studied separately at a time while other factors are held constant. This can lead to erroneous conclusions about the importance of certain factors on the extraction process, due to the fact that interactions between factors are not being considered. A multivariate optimisation procedure that allows

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simultaneous variation of all experimental factors has become popular. This enables the investigation of main effects as well as interaction effects influencing the SPME process (Polo et al., 2005).
The objective of this work was to identify the SPME fibre type with highest extraction efficiency as well as to investigate the main and interaction effects influencing the HS-SPME process for the simultaneous analysis of the four key odorants in pit latrines. The goal of this work was that the optimised method would be then applied for analysis of microbial deodorisation of butyric acid, DMTS, indole and p-cresol using bacterial strains that had been isolated and identified in our laboratory.

2. Experimental

2.1. Reagents and materials

All chemicals used in this study were of analytical grade. Ethanol (≥99 %), Butyric acid (99 %), Hydrochloric acid (32 %) and sodium chloride were purchased from Merck Chemical (Pty) Ltd, Gauteng, South Africa. DMTS (≥98 %), indole (≥99 %) and p-cresol (99 %) were purchased from Sigma Aldrich Inc., St Louis, MO, USA. Ultrapure water was prepared by Direct Q Millipore purification system (Merck Millipore, Germiston, South Africa.
The SPME manual holders and fibres were obtained from Supelco (Bellefonte, PA, USA). In this work five commercially available fibres used were; 24 ga and 1 cm fused silica/SS 85 µm Polyacrylate (PA) and 100 µm Polydimethylsiloxane (PDMS), 24 ga stableflex/SS 85 µm CAR–PDMS, 65 µm polydimethylsiloxane–divinylbenzene(PDMS–DVB) and 50/30 µm Carboxen–Divinylbenzene Polydimethylsiloxane (CAR/DVB/PDMS). The fibres were conditioned as recommended by the manufacturer.

2.2. Sample Preparation

The stock solution was prepared by dissolving the solid/ liquid standards in ethanol containing 10,000 $\mu g mL^{-1}$ each of pure analytes of butyric acid, DMTS, indole and p-cresol and stored in darkness at 4 ^{o}C. The working solutions of the standards were also serially diluted in ultrapure water to a concentration of 0.5 mgL^{-1}.

2.3. HS-SPME Procedure

A sample (10 mL) 0.5 mg L− 1 of DMTS, butyric acid, p-cresol and indole was placed in a sealed 20 mL amber glass vial with 5 g of NaCl. A small magnetic polytetraflu-orothylene (PTFE) - coated stirring bar was also added. The pH of the sample was adjusted to 2. The vial was tightly closed with a PTFE-coated silicone septum. The vial was placed in a 200 mL beaker filled with 100 mL of water and then put on a thermostatted block with a stirrer. The sample was incubated for 20 min at extraction temperature of 40 ^{o}C and, after this, the SPME fibre was inserted into the headspace for extraction time of 30 min while the sample was stirred at a constant rate of 800 rpm. After extraction, the fibre was removed from the sample vial and inserted into inserted into the injection port of the GC for desorption. To avoid cross-contamination the SPME fibre were preconditioned for 10 min at the same desorption temperature.

2.4. GC/TOF-MS analysis conditions

The analysis was carried out with an Agilent 7890A Gas Chromatograph (GC) system (Agilent Technologies, Palo Alto, CA, USA) coupled with Pegasus 4D Time of Flight Mass Spectrometry system (LECO Corp, St Joseph MI, USA). Separation of the extracted components was performed on a 30 m x 0.25 ID fused silica capillary column (Restek Corp. Bellefonte, PA, USA) having a 0.25 µm film thickness of Stabilwax (Crossbond®Carbowax®polyethylene glycol). The injection port equipped with SPME Borosilicate Glass specifically designed narrow straight liner of 0.75 mm ID and deactivated by the manufacturers (Restek Corporation, USA) to increase linear velocity and introduce analytes onto the GC column in a narrow band, thus leading to the sharper peak of the analytes (Sigma Aldrich., 1997).
The SPME fibre was manually injected and the sample extracts were thermally desorbed in the slit/splitless injector that was operated in splitless mode for 5 min at an inlet injector temperature of 250 ^{o}C and then, it was baked at the same temperature for 10 min. The GC column oven temperature program was initially held at 40 ^{o}C for 1 min, then a ramp of 30 $^{o}C\ min^{-1}$ to 100 ^{o}C and then ramp of 15 $^{o}C\ min^{-1}$ to 200 ^{o}C and then ramp of 20 $^{o}C\ min^{-1}$ to 240 ^{o}C and then held for 3 min. The total run time for the analysis was 14 min 37 s. This was established on the basis of the complete separation obtained for all sample components using the standard solutions. The carrier gas used was ultrahigh purity (UHP) helium at constant flow rate of 1.0 mL min^{-1} with the column head pressure of 7.192 psi. The ion source and transfer line temperatures were maintained at 230 ^{o}C and 240 ^{o}C. The TOFMS detector was operated in the electron impact (EI) mode with an electron ionisation voltage of -70 eV at an acquisition rate of 20 spectra/s in full scan within the range of 35 to 450 m/z detected in total ion current (TIC) mode.

The electron multiplier was set to an auto tune procedure. A solvent delay time of 3 min 10 s was used to avoid overloading the mass spectrometer with ethanol. The system was operated by LECO ChromaTOF 4.50 data acquisition and processing software (LECO Corp, St Joseph MI, USA).

2.5. Data processing and analysis

TICs obtained were processed using automated peak find at S/N threshold of 100 with a library search in normal and forward mode. Components identification was based on comparison of both the retention times and mass spectra with those of the Willey 275 and NIST (USA) 92-Mainlib and Replib Mass spectral libraries on the full spectra generated from the authentic standards with the similarity percentage of at least 65 % under the identical experimental conditions. The experimental design and analysis of variance (ANOVA) of the collected data was done using Minitab statistical software (Release 17; Minitab Inc., PA, USA).

3. Results and Discussion

3.1. Selection of optimal SPME fibre

The sensitivity and selectivity of SPME extraction technique depends primarily on the value of the distribution constant for analytes partitioned between the sample matrix and fibre coating material dependent upon its type of stationary phase and also on its polarity and thickness (Risticevic et al., 2010). Therefore, fibre coating type is one of the key factors in the extraction efficiency to analytes.
For this reason, in this study the extraction efficiency of the five commercially available SPME fibre coatings described in Section 2.1 were evaluated for their extraction efficiency of the targeted odorous compounds in order to select the fibre coating for this application. Each fibre was exposed to the headspace under same condition of 20 min of equilibrium time, 50 ^{o}C of extraction temperature, 30 min. of extraction time, ionic strength at NaCl saturation point (500 mg mL^{-1}), sample volume of 10mL in 20 mL amber glass vial, constant stirring rate of 800 rpm and pH of 2.

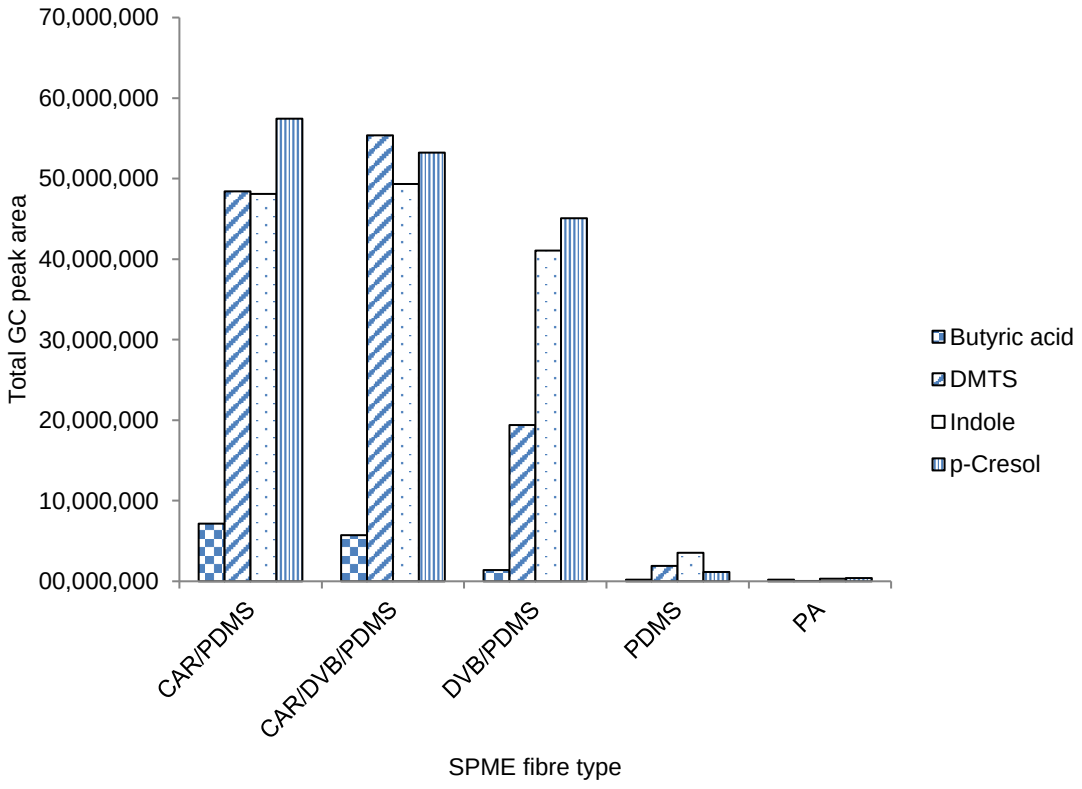

Figure 1: Performance characteristics obtained for tested SPME fibres

The differences in the GC peak areas obtained exposed the behaviour of each type of fibre coating used for each fibre in respect to their extraction capacity of the compounds under investigation. The results are presented

in Figure 1. As a result, it was found that CAR/PDMS/DVB and CAR/PDMS fibre coatings showed much higher extraction efficiency for the compounds under investigation than the other PDMS/DVB, PDMS and PA fibre coatings. However, on one hand CAR/PDMS/DVB had higher extraction efficiency for DMTS and indole than CAR/PDMS. CAR/PDMS had much higher extraction efficiency for butyric acid and p-cresol compared to all the evaluated fibre coatings. High sensitivity of these fibre coatings also showed much better repeatability (data not shown). CAR/PDMS has been reported that it does not favour production of oxidation products at high temperatures (Pia Gianelli, 2002). On the basis of the overall performance as shown in Figure 1, the CAR-PDMS was selected for the subsequent optimisation experiments.

3.2. SPME parameters optimisation

Based on the preliminary studies (data not shown) that were performed prior to optimization, four parameters that were selected for SPME optimization were extraction temperature, extraction time, sample pH and ionic strength (NaCl concentration).

Table 1: Factor levels in the designs for SPME optimisation

Factor(units)	Low (-)	Centre (0)	High (+)
A: Extraction temperature (°C)	40	55	70
B: Extraction time (min)	20	25	30
C: Sample pH	1	1.5	2
D: NaCl concentration (mg/mL)	0	250	500

The factors and their levels (low (−), centre (0) and high (+)) are defined in Table 1. The sample volume of 10 mL, equilibrium time of 20 min., constant stirring rate of 800rpm, was kept constant for all experiments. The effect of these factors from a low level to a high-level value was investigated on response such as total peak area of DMTS, butyric acid, p-Cresol and indole.

3.3. Screening by 2^4 full factorial design

The initial screening design was done to identify the factors which had main effects and interaction effects on the responses of each of the targeted compounds (DMTS, butyric acid, p-cresol and indole). The data generated from these parameters were evaluated by analysis of variance (ANOVA) at 95 % confidence level. All factors which had p-value of equal to or less than 0.05 are statistically significant while those with greater than 0.05 are statistically insignificant.

Table 2: Results of ANOVA indicating the statistical significance of main effects and their interactions

Compound		Factors				Interactions					
		A	B	C	D	AB	AC	AD	BC	BD	CD
Butyric acid	F-ratio	105.340	11.040	0.010	151.040	5.640	0.520	43.720	0.280	7.050	1.870
	p-value	0.000	0.003	0.904	0.000	0.026	0.477	0.000	0.600	0.014	0.183
DMTS	F-ratio	0.040	0.060	0.080	1.230	0.210	3.040	2.700	0.390	0.290	0.040
	p-value	0.835	0.810	0.785	0.277	0.649	0.093	0.113	0.537	0.597	0.840
Indole	F-ratio	762.490	70.360	0.170	1205.340	5.170	1.480	187.300	1.120	14.920	5.470
	p-value	0.000	0.000	0.683	0.000	0.032	0.236	0.000	0.301	0.001	0.028
P-cresol	F-ratio	605.160	50.770	5.800	1197.150	4.590	0.070	156.800	0.230	14.530	0.000
	p-value	0.000	0.000	0.024	0.000	0.042	0.801	0.000	0.636	0.001	0.950

The results obtained were presented in Table 2. The results for DMTS showed that none of the factors and two-way interactions were statistically significant on the response values (total peak areas) at $\alpha = 0.05$. The results for butyric acid showed that the factors; extraction temperature, extraction time and NaCl addition (ionic strength) were statistically significant at $\alpha = 0.05$. Their two-way interactions of extraction temperature and extraction time, extraction temperature and ionic strength, and extraction time and ionic strength were also statistically significant at $\alpha = 0.05$.

The results for p-cresol showed that all the factors and their interactions of extraction temperature and extraction time, extraction temperature and ionic strength, and extraction time and ionic strength were statistically significant at $\alpha = 0.05$. The results for indole showed that factors; extraction temperature, extraction time and

ionic strength and their interactions of extraction temperature and extraction time, extraction time and ionic strength, and sample pH and ionic strength were statistically significant at $\alpha = 0.05$.

The effects for all compounds but DMTS indicate that ionic strength (NaCl concentration) was the most important factor, indicating statistical positive significance for butyric acid, p-cresol and indole. With reference to extraction temperature, it was shown that it was the second most important factor for HS-SPME process, it was statistically positive significant for butyric acid, p-cresol and indole. Extraction time also presented statistical positive for the three compounds (butyric acid, p-cresol and indole), whereas sample pH was statistically negative significant for only p-cresol.

This means that the higher the sample pH as a factor involves a significant decrease of chromatographic peak areas of p-cresol while for the other compounds had no significant effect on their chromatographic peak areas. Subsequently, this insinuates that the sample pH should be kept at low value only for deionization of butyric acid to enhance its affinity for the SPME fibre.

With reference to two-way interactions, that between extraction temperature and ionic strength (AD), extraction time and ionic strength (BD) and extraction temperature and extraction time (AB), in that descending order of magnitude of importance, were statistically positive significant for butyric acid, p-cresol and indole, whereas interaction between sample pH and ionic strength (CD) was statistically positive significant only for indole.

4. Conclusions

In this study, SPME fibre and main factors and their two-way interactions for the simultaneous determination of butyric acid, DMTS, p-Cresol and indole were identified. CAR/PDMS was selected as the fibre which gave the best overall extraction efficiency for these odorants. All the factors and their interactions were statistically insignificant at $\alpha = 0.05$ on the total peak areas of DMTS.

Extraction temperature, extraction time and ionic strength and their two-way interactions of extraction temperature and extraction time, extraction temperature and ionic strength and extraction time and ionic strength were statistically significant at $\alpha = 0.05$ on the total peak areas of butyric acid, p-Cresol and indole while sample pH and the interactions of sample pH and ionic strength were statistically significant for indole only. Further investigation needs to be done to find the optimal conditions for optimisation of HS-SPME-GC/TOFMS for analysis of pit latrine key odorants.

This study became useful and effective input to efforts aimed at neutralising pit latrine odours, and if achieved would enhance the adoption and usage of pit latrines, consequently helping in achieving Sustainable Development Goal (SDG) 6.

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