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Short communication

Vapor-phase analysis of isobutyl acetate, isopropyl acetate, *n*-propyl acetate and their respective alcohols using solid-phase microextraction–gas chromatography with a mass selective detector

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Abstract

A solid-phase microextraction (SPME)–GC–MS method for three esters and the corresponding alcohols was tested for responses in accuracy, within-run precision (repeatability), and between-run precision (reproducibility) due to individual operators, individual analysis days, and differing analyte concentrations. At 5 ppm (v/v) [ppmv], three of the six analytes showed significant ($p < 0.05$) operator effects, while five of six analytes gave a significant effect due to the days of analysis. At 20 ppmv, five of the six analytes gave significant operator and daily effects. At 100 ppmv, all the analytes showed significant daily effects but no operator effects were observed. The repeatability was concentration dependent, with all six analytes combining for an average RSD of 12.1 ± 6.1% at 1 ppmv, becoming most precise at 50 ppmv at 1.01 ± 0.45%, then increasing at 100 ppmv to 4.12 ± 1.88%. The contributors to error trended as: concentration > daily effects > operator.

Keywords: SPME; GC–MS; Volatiles; Soil gas; Ester; Alcohol

1. Introduction

Solid-phase microextraction (SPME) for the analysis of volatile analytes has many advantages and is well established. SPME coupled with GC–MS provides a powerful investigative and quantitative tool which has been employed for many diverse disciplines including the analysis of volatile constituents in air [1,2], flavors [3–5] and volatiles from pharmaceuticals [6], plants [7], fungi [8–10], and bacteria [11,12]. By screening volatiles from these sources, a wide range of compounds may be found and quantified.

The between-run precision (reproducibility) and within-run precision (repeatability) [13] of SMPE–GC–MS has been questioned for reasons including fiber to fiber variation [14,15], matrix effects [3,5,14], fiber aging [14], temperature variations [16], and general changes in experimental conditions [3]. For example, one group reported that "poor reproducibility typically plagues SPME," in a headspace study quantifying derivatized tributyltin [17]. In response, they then developed an isotope dilution method which improved the reproducibility. Because individual analytes have different partitioning properties, using isotope dilution for each analyte should provide the best quality data possible, but this practice would need to be weighed against the increased costs.

Day to day effects on SPME precision have been quantified and reported. Using an ion trap MS and a polydimethyl siloxane (PDMS) fiber, with seven replicate injections, the mean relative standard deviation (RSD) for eight compounds was 2.3% for a given day, and increased to 3.1% for the pooled data by one analyst on the same instrument over three consecutive days [6]. In this system there was little difference between repeatability and reproducibility, in contrast to other reports. Consistent stirring was reported to be one of the most important factors for better precision.

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A study on flavor analysis using PDMS with GC–MS reports the RSD ranged from 0.5% for phenylethyl alcohol to 18.3% for triacetin and 17.8% for ethyl acetate [3]. The authors of this study observed an average RSD of 7% is generally acceptable in trace organic analysis, revealing that of the 22 compounds reported, 7 exhibited an unacceptably high variation. This is similar to the repeatability reported using a polyacrylate fiber, where the average RSD was 11% for acetone, ethanol, and 13 fatty acids [4].

The repeatability using SPME is reportedly better than charcoal tubes. It was found that charcoal tubes (National Institute for Occupational Safety and Health [NIOSH]-1550) RSDs ranged from 16 to 41% for C_5–C_{15} alkanes, compared to 2–6% for 100 μm PDMS coating SPME sampling [1]. In comparisons using benzene, toluene, ethylbenzene and xylene (BTEX) compounds, the accuracy was not significantly different in charcoal versus SPME, while SPME was more precise, resulting in RSDs of 1.6, 3.8, 3.9, and 4.8%; much tighter than the corresponding charcoal tube 5.0; 6.3; 7.1; and 19% RSDs, respectively, for benzene, toluene, ethylbenzene, and p-xylene standards. There was a minimum 10-fold reduction in sampling time for air monitoring of SPME versus NIOSH charcoal tubes.

Further, a manual headspace SPME method has been compared with an automated static headspace method for alcohols and esters in beer, using 1-pentanol as an internal standard [19]. Manually using polyacrylate SPME with GC-flame ionization detection (FID), the RSD in prepared standards ($n = 7$) ranged from 1.80 to 10.80%, with a mean of 5.5%. Similarly, the automated static headspace method ranged from 1.3 to 10.0% RSD, with a mean of 3.1%. In a beer matrix, the manual SPME method again compared closely, with a range of 0.31–6.8% and a mean of 3.0% ($n = 3$), compared to the automated static headspace method, which produced a RSD range of 0.32–10.2%, with a mean of 2.5%. The repeatability of the manual SPME method in this report therefore compares closely with the automated headspace method.

Rocha et al. [5] found similar precision in an investigation on the effect of matrix volatile composition on relative response factors (RRFs) of flavor components in a wine model using polyacrylate SPME fibers. The RSD ranged from 1.5% for 3-methyl-1-butanol to 12% for ethyl octanoate. Interestingly, a temporal replacement effect was found, with ethyl decanoate displacing both ethyl octanoate and ethyl hexanoate.

Namiesnik et al. [16] reported that accuracy is effected by the temperature and humidity of the SPME binding matrix. This is important in that the precision may not be affected, but the accuracy might. The group reports that compared to dry SPME sampling, at 92% humidity (20 °C) there were quantification losses about 70% for chlorobenzene, 60% for toluene and *p*-xylene, and 30% for CCl_4 and *n*-decane. This factor may not plague precision but might become a problem with accuracy in gas samples from the field, as gas calibration standards are typically made dry, while the environmental samples may have large differences in humidity.

From these studies, it becomes apparent that finding the unique variation for each specific application and possibly for each run would be a good practice. The analytical reproducibility and repeatability of three small esters and their associated alcohols is the focus of this paper. We developed a method for monitoring soil gases in a subsurface remediation application. Specifically our goal was to develop a method to quantify three small esters and their corresponding alcohols routinely, yet leave room for qualification and further quantification of TICs in the soil gas samples. As such, a SPME preconcentration was performed before analysis by scanning GC–MS. In our field remediation system, we predicted a need to analyze higher concentrations of esters than the corresponding alcohols, and therefore chose polyacrylate (PA) fibers for the increased sorption of alcohols compared to PDMS fibers [20], while still being acceptable for esters, lessening potential abundance problems in the analysis. The PA fiber sorbed for 15 min is reportedly optimal for precision over a range of volatiles [21]. Carboxen SPME fibers were used occasionally for qualitative screens, but were not used for quantification due to the inherent uncertainty of matrix effects in our field samples, the competition for sites on this style of fiber, and the high expected concentrations of all analytes expected in our application. We statistically tested our data to determine if individual operators and daily intra-operator variances effected quantification of several volatile compounds over a 4-day interval. Concentration dependent effects on repeatability were assessed, and the reproducibility of the method over six months is also presented.

2. Experimental

2.1. SPME

The 85 μm polyacrylate fibers (Supelco no. 57318) with a manual SPME fiber holder (Supelco 57330-U) were used with a 15 min sorption time at room temperature and a 2.0 min desorption time at 280 °C. The high inlet temperature was chosen to ensure the recovery of heavier volatile and semivolatile compounds, to complement the scanning MS detection system for the detection of a wide range of analytes [22–24]. Initially, we determined that target analytes were stable under these conditions.

2.2. GC–MS

We used an Agilent 6890 Series GC System with an Agilent 5973 Network Mass Selective Detector, and an HP-624 Special Analysis Column (HP19091V-402 capillary 25.0 m × 200 μm ID × 1.12 μm nominal). Helium was the

Table 1
Retention times and quantified ions of the target analytes

	Isopropyl alcohol	*n*-Propyl alcohol	Isobutyl alcohol	Isopropyl acetate	*n*-Propyl acetate	Isobutyl acetate
t_R (min)	3.80	5.12	6.74	6.97	8.32	9.84
Quantified ions						
Target (amu)	45	59	43	43	43	43
Q1 (amu)	43	42	41	61	61	56
Q2 (amu)	–	60	–	87	73	73

carrier at 27 cm/s average velocity, the temperature program began with a 2 min hold at 40 °C, ramping to 110 °C at 8 °C/min and holding 2 min, then ramping at 20 °C/min to 180 °C and holding an additional 5 min, for total run time 21.25 min. A 0.75 mm ID injection sleeve (Supelco no. 2-6375) was used with a split ratio of 20:1 and a split flow rate of 9.5 mL/min for a total flow 12.9 mL/min (gas saver off). The quantified ions and retention times of the analytes are listed in Table 1.

2.3. Standards

Gas standards were prepared by dilution with UHP nitrogen in 2.0 L, 9 in. × 9 in. Tedlar bags (Alltech no. 41049, Deerfield, IL) using an initial standard from Norlab (Boise, ID) containing 100 ppm (v/v) [ppmv] propyl acetate, 99.8 ppmv isopropyl acetate, 99.7 ppmv isobutyl acetate, 100 ppmv propyl alcohol, 100 ppmv isopropyl alcohol, and 99.7 ppmv isobutyl alcohol with the balance nitrogen.

Table 2
Method performance from six replicate injections with the same polyacrylate fiber by two independent operators on four separate days, arithmetic means and variation in RSD; two-way nested ANOVA tests for operator and daily effects

Day	Operator	Detector response: mean (RSD as %)					
		Isopropyl alcohol	*n*-Propyl alcohol	Isobutyl alcohol	Isopropyl acetate	*n*-Propyl acetate	Isobutyl acetate
Level: 5 ppmv							
1	1	4374 (18.3)	1736 (9.28)	9837 (3.76)	9085 (15.0)	20,816 (9.94)	31,125 (6.23)
2	1	4794 (5.37)	1881 (3.46)	8365 (3.15)	10,740 (2.82)	21,744 (3.50)	29,202 (4.19)
3	2	5964 (3.30)	2252 (3.95)	10,087 (4.31)	12,822 (4.06)	26,134 (4.26)	34,490 (4.32)
4	2	6112 (4.70)	2391 (5.11)	10,186 (3.26)	13,359 (3.60)	28,279 (2.83)	37,649 (2.67)
1, 2, 3, 4	1, 2	5311 (16.4)	2065 (14.2)	9619 (8.53)	11,501 (16.4)	24,243 (13.9)	33,117 (10.8)
Variance source	Statistic						
Operator	*p*-value	0.0226	0.0364	0.2958	0.0674	0.0367	0.0856
Operator	Percent of total variance	82.3	85.7	28.8	78.6	85.9	75.2
Day	*p*-value	0.2629	0.0231	0.0001	0.004	0.019	0.0012
Day	Percent of total variance	1.2	5.4	57.3	11.0	5.5	14.7
Level: 20 ppmv							
1	1	19,555 (6.10)	7684 (2.94)	38,647 (3.65)	37,620 (5.09)	80,429 (3.37)	119,958 (2.97)
2	1	17,566 (2.46)	7258 (1.39)	31,303 (2.31)	35,270 (2.27)	77,589 (2.15)	109,020 (2.00)
3	2	25,377 (1.35)	10,417 (2.84)	43,925 (1.86)	53,017 (1.68)	114,536 (3.08)	158,230 (2.16)
4	2	24,260 (1.55)	10,054 (2.91)	41,592 (2.22)	51,132 (1.18)	112,290 (2.17)	154,674 (1.39)
1, 2, 3, 4	1, 2	21,698 (15.5)	8854 (16.3)	38,867 (12.7)	44,260 (18.4)	96,211 (18.5)	135,470 (16.2)
Variance source	Statistic						
Operator	*p*-value	0.0316	0.0101	0.1808	0.0092	0.0028	0.0183
Operator	Percent of total variance	91.8	96.8	59.3	97.3	98.5	95.6
Day	*p*-value	0.0001	0.0028	0.0001	0.001	0.0873	0.0001
Day	Percent of total variance	5.9	1.7	38.1	1.6	0.3	3.5
Level: 100 ppmv							
1	1	102,005 (7.57)	42,175 (5.86)	203,963 (5.80)	190,957 (5.45)	413,934 (4.47)	639,564 (3.84)
2	1	84,692 (2.76)	35,135 (2.08)	152,223 (3.01)	166,059 (2.24)	360,239 (2.18)	514,614 (2.42)
3	2	93,278 (3.40)	38,782 (3.13)	165,425 (3.68)	187,451 (1.30)	414,760 (1.91)	585,265 (2.00)
4	2	112,474 (1.97)	46,891 (1.50)	197,989 (1.89)	229,646 (1.47)	505,438 (2.06)	715,096 (1.94)
1, 2, 3, 4	1, 2	98,112 (11.5)	40,746 (11.4)	179,900 (12.9)	193,438 (12.4)	423,593 (12.9)	613,635 (12.5)
Variance source	Statistic						
Operator	*p*-value	0.5378	0.5176	0.9167	0.3412	0.3001	0.5024
Operator	Percent of total variance	0	0	0	20.3	30.6	0
Day	*p*-value	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Day	Percent of total variance	89.1	93.0	94.6	75.3	70.0	96.7

The final volume was 1 L in the 2 L bags, leaving room for mixing by hand.

2.4. Validation

Initially, standards of 5, 20 and 100 ppmv were injected six times each by two individual operators on four different days, with an MS autotune at the beginning of each day. Nested ANOVA was used to estimate the contribution of operator and day effects to the overall variation in instrument response. Analyses were conducted using SAS/STAT software, Version 8 of the SAS System for Windows (SAS Institute Inc., Cary, NC, USA).

Running standard curves for field sampling were performed using single injections of 1, 5, 20 and 100 ppmv preparations over 9 weeks. The MDLs were estimated as per 40 CFR 136, Appendix B, with nine replicate injections of 1 ppm of a mix of all six standards in nitrogen.

3. Results and discussion

The means and relative standard deviations varied by day and by concentration (Table 2). Within individual days, the variation ranged from 1.18 to 18%. Isobutyl alcohol gave a daily RSD that ranged from 1.86 to 5.80%. When the 4 days of data for isobutyl alcohol were pooled, the RSD was 11.4%. This shows the daily repeatability was considerably (3.5×) tighter than the reproducibility over the 4 days. The pooled isobutyl alcohol repeatability was similar to the precision found between individual fibers and within one fiber, which had intra-fiber RSDs of 9.4, 10.1, and 11.9%; and an inter-fiber (combined) RSD of 10.4% [15]. The system gave 1.91–9.94% repeatability and 12.9–18.5% reproducibility for *n*-propyl acetate. This is comparable with the previously reported RSD for *n*-propyl acetate of 12.0 and 5.1% for two separate fibers [18].

All but two of the comparisons showed daily effects (Table 2). In contrast, significant operator effects (*p*-values) were observed in three of six analytes at 5 ppmv, five of six at 20 ppmv, and none at 100 ppmv. This difference in operator error between concentrations possibly reflects variances manifested during the production of the standards. At 100 ppmv the bags were simply inflated with 100 ppmv from a standard tank. The 5 and 20 ppmv standards were made fresh by diluting the 100 ppmv standard. Therefore, operator errors at concentrations requiring dilution might be due to errors in making the standards as opposed to error generated from the SPME–GC–MS procedure. The distribution of the total variance agrees with this concentration dependent finding (Table 2).

The esters gave the best repeatability from 50 to 80 ppmv while the corresponding alcohols gave the best results at 10–20 ppmv (Fig. 1). Rocha also used polyacrylate SPME fibers with GC-FID quantification and found a similar loss of reproducibility at the low concentrations tested, but with-

Table 3
Data from standard curves spanning a period from 11/27/02 to 6/6/03 ($n = 9$)

Pooled							
Mean	1 ppm STD	5 ppm STD	20 ppm STD	100 ppm STD	Slope	*y*-intercept	rsq
n-Propyl alcohol	172	2009	8074	39058	391	14	0.99988
Isopropyl alcohol	850	5381	20559	95066	946	671	0.99973
Isobutyl alcohol	1556	9729	38706	184660	1843	616	0.99989
Isopropyl acetate	2411	10565	40268	189037	1879	1383	0.99989
n-Propyl acetate	3902	22963	85968	404074	4021	2569	0.99984
Isobutyl acetate	5511	33108	127937	616690	6152	2009	0.99994

Pooled					Running standard curves: *y*-intercept		
STDEV	1 ppm STD	5 ppm STD	20 ppm STD	100 ppm STD	Max	Min	Mean
n-Propyl alcohol	186	672	2738	12033	780	−533	16
Isopropyl alcohol	439	1722	7163	29550	3024	−386	745
Isobutyl alcohol	737	3401	15431	59788	6240	−1997	684
Isopropyl acetate	1703	2401	10741	49310	3732	−868	1537
n-Propyl acetate	1406	5203	21737	106516	7235	−971	2854
Isobutyl acetate	1886	7762	35091	168145	8920	−3917	2232

Pooled					Running standard curves: slope			
RSD	1 ppm STD	5 ppm STD	20 ppm STD	100 ppm STD	Max	Min	Mean	%RSD
n-Propyl alcohol	108	33	34	31	640	283	434	28
Isopropyl alcohol	52	32	35	31	1514	715	1051	28
Isobutyl alcohol	47	35	40	32	3015	1402	2048	29
Isopropyl acetate	71	23	27	26	2925	1551	2088	24
n-Propyl acetate	36	23	25	26	6499	3045	4468	24
Isobutyl acetate	34	23	27	27	9974	4926	6836	25

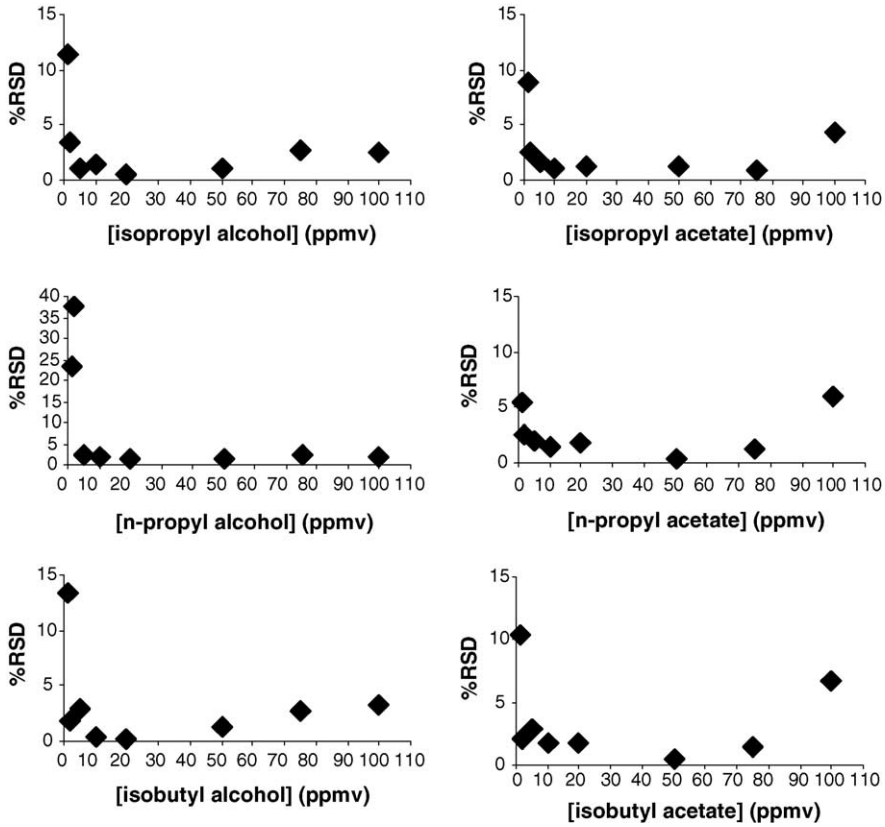

Fig. 1. The RSD as a function of concentration, note the *y*-axis difference for *n*-propyl alcohol. There were three replicates of each concentration with the exception of the lowest standard, 1 ppm, which received nine replicate injections. There was not a downward trend in the nine replicate injections; i.e. the loss of repeatability was not due to analyte removal.

out a loss at the upper concentrations [5], this difference is possibly attritutable to the increased linear range of FID over MS.

Means of pooled data from the running standards over 8 weeks show that data are linear (Table 3). The loss of repeatability of the esters at the higher concentrations (Fig. 1) is therefore likely not an effect attributable to going above the linear calibration range. At the lower end, the 1 ppm standards show higher RSD values compared to the RSD values for 5–100 ppmv. This suggests that 1 ppm is approaching the instrument detection limit, and indeed the MDLs were 0.41 ppmv for *n*-propyl alcohol; 0.27, isopropyl alcohol; 0.67, isobutyl alcohol; 0.21, isopropyl acetate; 0.13, *n*-propyl acetate; and 0.25 for isobutyl acetate.

4. Conclusions

Using identical procedures, a single newly purchased and conditioned fiber, the same Tedlar bags, the same tank of standards, identical handling procedures, and the same hardware, there were still significant differences between individual days and also between individual operators. The differences found between operators may be partially attributable to the dilution of the standards in the lower concentrations, and in all cases were not predictable.

The RSD for within-run precision (repeatability) averaged 5.5 ± 3.9, 2.5 ± 1.2, and $3.1 \pm 1.7\%$ for 5, 20, and 100 ppmv, respectively, of the six analytes, compared to 13.4 ± 3.1, 16.3 ± 2.1, and $12.3 \pm 0.7\%$ for the between-run precision (reproducibility) for the three concentrations. Overall, the precision of SPME–GC–MS in this study compared closely to the typical 2–10% [25] precision of quantitative MS, and within the 15% requirement presented in Environmental Protection Agency (EPA) Method SW846-8260B. In this study as well as in previous reports, the precision was difficult to predict and account for, and tended to change considerably over time, sample sets, compounds tested, and operators. The contributors to error trended as: concentration > daily effects > operator.

Using SPME–GC–MS for screening a wide range of volatiles is powerful, however method validation using this system should be approached carefully, particularly considering that the number of samples required to achieve a given confidence is in proportion to the square of the empirically derived variation. Although currently not available in a system compatible for use with Tedlar bags, automated sampling should eliminate some of the indeterminate error, as should the use of appropriate internal standards, particularly using

isotopic dilution [17]. Even so, because of the degree of complexity in the total system, it may be beneficial to run a series of matrix spiked samples daily to estimate the complete system variability if the sample priority is high, and particularly if the matrix is expected to vary.

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