

Evaluation of Septa Quality for Automatic SPME–GC–MS Trace Analysis

Agnieszka Ulanowska¹, Tomasz Ligor¹, Anton Amann^{2,3} and Bogusław Buszewski^{1*}

¹Chair of Environmental Chemistry and Bioanalytics, Faculty of Chemistry, Nicolaus Copernicus University Gagarin Street 7, 87-100 Toruń, Poland, ²Univ.-Clinic of Anesthesia, Innsbruck Medical University, Anichstrasse 35, 6020 Innsbruck, Austria, and ³Breath Research Institute, Austrian Academy of Sciences, Dammstrasse 22, A-6845 Dornbirn

*Author to whom correspondence should be addressed: email bbusz@chem.uni.torun.pl.

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The vials used for the preparation of breath samples for automated solid-phase microextraction–gas chromatography–mass spectrometry analysis are crimped with septa. These septa often emit specific volatile organic compounds (VOCs) confounding the measurement results of breath samples. In the current paper, 14 different brands of magnetic caps with silicone–polytetrafluoroethylene (PTFE), butyl–PTFE, or butyl rubber septa were tested. The total emission of septa over a 4 h period was also evaluated. The tested septa emitted 39 different compounds, which are mainly hydrocarbons, alcohols, and ketones. Acetone and toluene are the most abundant out-gassing products. The concentration of acetone was in the range from 55 to 694 ppb for butyl–PTFE septum (brand 14) and butyl rubber (brand 10), respectively. The measured toluene amount was 69–1323 ppb for the septum brand 14 and brand 8 (silicone–PTFE), respectively. Generally, the butyl rubber septa released higher amounts of contaminants in comparison to the silicone ones.

Introduction

The determination of trace gas components in samples with complex and varying matrix compositions is demanding. The final result of trace gas analysis is influenced by many factors, such as sampling, storage, sample pretreatment, separation of constituents, detection, and quantification. Negligence with respect to one of the mentioned steps may become a source of errors that makes the measured data useless (1). The biggest error can be made during sampling and sample preparation (60%), measurement and calibration (30%), and during data processing (10%).

Volatile organic compounds (VOCs) identified in natural samples (e.g., exhaled air, headspace of bacteria, and tissue) are present in trace concentrations (ppbv/ppbv level) (2–4). Therefore, for detection of the analytes, different methods for pre-concentration and enrichment are usually applied. Thermal desorption (TD) and solid-phase microextraction (SPME) are the most often used techniques for this purpose (5, 6).

Gaseous samples for automatic SPME process are usually stored in glass halogenated solvent (HS) vials closed with crimp or screw caps equipped with a septum. Septa might release different substances that are detected during sample analysis. Additionally, the emission of contaminations can interfere with the investigated or potentially important compounds. Septa are typically made of butyl rubber or silicone. Butyl rubber is airtight and seals well (7). This polyisobutylene polymer consists of 98% of isobutylene with approximately 2% of isoprene. Therefore, butylene-derivatives might be emitted

by those septa. They also release COS and CS₂ (8). Septa intended for covering of analytical samples are coated by polytetrafluoroethylene (PTFE, Teflon). Teflon coating is applied to reduce negative factors such as: permeation through the septum, absorption of the analytes, and the off-gassing process. Additionally, PTFE is a chemically inert material that does not react with analytes (9). They can be monomers, depolymerization, or ageing, and organic solvents applied during the technological process. The silicone–PTFE septa are widely used because this material is temperature resistant (the maximum service temperature is 225°C) and soft and easy to puncture by needle even if tapered. In general, silicone septa display less off-gassing product (10–12). This kind of septum was used for sample preparation before SPME–GC–MS analysis of explosives in soils (13). Therefore, it is often preferred. On the other hand, butyl rubber–PTFE septa are mechanically hard and more resistant to the absorption of the constituents of a sample, and it is stable up to 130°C (12, 14). Kolb et al. showed that in using silicone–PTFE rubber septa, 69% of acetone and 89% of dichloromethane were lost, while in butyl–PTFE rubber septa, the losses were less than 3%. This outcome indicated that butyl rubber is denser and less permeable than silicone ones (15).

In the present work, 12 different brands of magnetic crimp caps with silicone–PTFE, butyl rubber–PTFE septum, and two different types of magnetic screw caps with silicone–PTFE membrane were tested. The evaluation of septum emission over a 4 h period was also examined. The goal of this investigation was the identification of VOCs emission from different kinds of HS septa and the quantitation of the most abundant compounds.

Experimental

Apparatus

The GC–MS analysis was performed on an Agilent 5975 Inert XL MSD, coupled with 6890 N gas chromatograph (Agilent Technologies, Waldbronn, Germany) with a split/splitless injector. The temperature of the injector was 200°C, and injections/SPME desorption were made in splitless mode. Helium was used as a carrier gas with a linear velocity of 40 cm/s. The MS analyses were carried out in a full scan mode, with a scan range of 15–220 amu. The scan rate was 3.46 scan per s. The electron impact ionization was used at energy 70 eV. The temperature of the ion source and the transfer line was 190°C and 150°C, respectively. The acquisition of the chromatographic data was performed by means of Chemstation Software

(Agilent). The 25 m × 0.25 mm × 3 μm capillary column, CPQ (Varian Inc., Middelburg, The Netherlands) was used. The oven temperature program was as follows: initially, 40°C held for 2 min, then ramped 10°C/min to 140°C, next ramped 5°C/min into 270°C and held for 3 min.

The auto sampler for the GC–MS system equipped with SPME Carboxen-PDMS coated (75 μm) fiber was purchased from Gerstel (Gerstel, GmbH & Co. KG, Germany). For the sample preparation, 20-mL HS glass vials (Perkin Elmer, Shelton, CT) were used. Gas tight syringes were bought from Hamilton (Hamilton, Reno, NV). The 1 dm³ Tedlar bags (SKC, Eighty Four, PA) were used for the breath samples collection. Twelve types of crimp caps with butyl rubber–PTFE or the silicone–PTFE septum and two types of screw caps with silicone–PTFE septum were tested. The details on the tested caps are presented in Table I.

The manual SPME holder with Carboxen-PDMS coated (75 μm) fiber was purchased from Supelco (Supelco Park Bellefonte, PA) and was used for the pre-concentration of VOCs in breath directly from a Tedlar bag.

Preparation of vials prior to SPME–GC–MS analysis

Before using the glass vials, they were flushed with argon and heated at 60°C for a few hours to remove any contaminants. The vials were purged for 3 min in a stream of pure nitrogen (5.0) and then crimped. Ten minutes after sealing, an SPME fiber was introduced to the vial for pre-concentration and to enrich the gases emitted by the septum. This extraction stage

was performed for 10 min at 30°C. Afterwards, a GC–MS analysis was performed. The analysis of outgassing from each septum was repeated 3 times.

Breath sample collection

Before human breath collection, the Tedlar bags were thoroughly cleaned by flushing with gas, and then filled with nitrogen and heated at 60°C for several hours to remove residual contaminants. The automatic breath sampler was used for the collection of alveolar breath and ambient air (Medical University of Innsbruck, Austria). This device enables exhaled breath sample collection in a CO₂-controlled manner. The first exhalation is used as a reference value and is never collected. During subsequent exhalations, the Tedlar bag was filled with alveolar breath. Afterwards, 10 mL of the breath sample was transferred from the Tedlar bag to a sealed glass vial.

Results and Discussion

The VOCs emitted (outgassing products) from various HS septa were tested. Altogether, 39 different compounds were identified. Emitted compounds belong to various chemical classes, such as alcohols, ketones, aldehydes, and hydrocarbons. Their contributions in total concentration were in the range of 2–10%, 5–35%, 1–5%, and 10–65%, respectively. Such emission can be attributed to the production process of septa material, such as the solvents, monomers, and additives. These compounds could also be formed during the processing of the septa. When two commercially available materials are compared, the butyl rubber septa releases higher amounts of contaminants than the silicone ones. The concentrations of the main VOCs found in septa emissions is presented in Table II.

Septum brand 7 (silicone/PTFE) gives the lowest emission of VOCs, and only 18 compounds were found. In contrast, the highest outgassing level was observed for brand 10 (butyl rubber). These septa present incredibly high emission of various compounds (i.e., the concentration of acetone exceeds 600 ppb). It shows that a PTFE covering decreases the VOCs emitted from the rubber.

Acetone and toluene are the most abundant outgassing products of commercially available headspace septa. In the case of the remaining septa, (brand 1–5, 9, and 13) the amount of toluene was in the range of 45–65% of the total emission. The observed concentrations of toluene ranged from 1323 ppb to 69 ppb for brand 8 (silicone–PTFE) and 14 (butyl–PTFE), respectively. The concentration of acetone was in the range of 65–694 ppb.

The emission of other compounds, such as ethanol, 2-methylpentane, and hexane was in the range 32–87 ppb, 16–93 ppb, and 17–36 ppb, respectively. In general, aldehydes, except etanal and propanal, were not found in outgassing products. However, the amount of acetaldehyde exceeds 200 ppb in a few cases. Among all released compounds, 2-methyl-1,3-butadiene (isoprene), which is one of the main metabolism products, was not found. All compounds identified in the emission from the tested septa are presented in Table III.

Table I
Properties of Tested Septa

No.	Competitor	Composition of septum	Additional information	Shore (°)	Thickness (mm)	Diameter (mm)
1	Brand 1	butyl/PTFE	crimp cap	-	3.0	20
2	Brand 2	silicone/PTFE	screw cap	35	1.3	18
3	Brand 3	silicone	Ultra Clean, screw cap	45	1.3	18
4	Brand 4	white/PTFE silicone	crimp cap	45	3.0	20
5	Brand 5	transp. blue/PTFE white silicone	Y-cut, crimp cap	55	1.3	20
6	Brand 6	white/PTFE red	crimp cap	55	1.3	20
7	Brand 7	silicone blue/PTFE grey	Ultra Clean, Pharma-Fix-Septa, crimp cap	45	3.0	20
8	Brand 8	silicone	Ultra Clean, crimp cap	45	3.0	20
9	Brand 9	transp. blue/PTFE white silicone	crimp cap	45	3.25	20
10	Brand 10	white/PTFE beige	crimp cap	55	3.0	20
11	Brand 11	butyl dark grey	crimp cap	50	3.0	20
12	Brand 12	butyl/PTFE grey	Pharma-Fix-Septa, crimp cap	50	3.0	20
13	Brand 13	butyl grey/PTFE grey	crimp cap	50	3.0	20
14	Brand 14	butyl grey/PTFE	crimp cap	-	3.0	20

Table II

The Most Often Identified Compounds and Their Concentration in PPB

Compound	Acetaldehyde	Ethanol	Butane	Acetone	Pentane	2-Methyl-pentane	3-Methyl-pentane	Hexane	Toluene
Brand 1 (butyl/PTFE)	6	44	12	94	6	16	20	22	329
Brand 2 (silicone/PTFE)	–	38	–	65	6	18	28	20	361
Brand 3 (silicone /PTFE)	–	37	11	69	6	16	16	18	310
Brand 4 (silicone/PTFE)	–	74	12	103	6	16	16	29	419
Brand 5 (silicone/PTFE)	–	49	13	95	6	16	16	17	329
Brand 6 (butyl/PTFE)	81	87	19	170	10	16	16	17	128
Brand 7 (silicone/PTFE)	15	33	–	85	–	17	16	14	82
Brand 8 (silicone/PTFE)	–	71	–	136	30	93	62	36	1323
Brand 9 (silicone/PTFE)	65	76	–	136	11	37	30	23	868
Brand 10 (butyl rubber)	262	50	13	694	43	57	62	20	97
Brand 11 (butyl/PTFE)	466	45	–	270	27	31	27	17	83
Brand 12 (butyl/PTFE)	250	35	–	184	7	16	17	17	103
Brand 13 (butyl/PTFE)	–	44	12	91	6	20	27	32	262
Brand 14 (butyl/PTFE)	–	32	–	55	8	19	25	21	69

Table III

All Compounds Identified in Septa Emission

	Brand 1 (butyl/ PTFE)	Brand 2 (silicone/ PTFE)	Brand 3 (silicone /PTFE)	Brand 4 (silicone/ PTFE)	Brand 5 (silicone/ PTFE)	Brand 6 (butyl/ PTFE)	Brand 7 (silicone/ PTFE)	Brand 8 (silicone/ PTFE)	Brand 9 (silicone/ PTFE)	Brand 10 (butyl rubber)	Brand 11 (butyl/ PTFE)	Brand 12 (butyl/ PTFE)	Brand 13 (butyl/ PTFE)	Brand 14 (butyl/ PTFE)
Acetaldehyde	+					+	+		+	+	+	+		
Acetone	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acetonitrile	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Benzene	+		+	+	+									
Butanal											+	+		
Butane	+		+	+	+	+				+			+	
2-Butanone											+	+		
2-Butenal										+				
Carbon disulfide						+				+	+	+		
Cyclohexane										+			+	+
Diethyl ether	+	+	+	+	+	+		+	+				+	+
2,3-Dimethyl-butane								+	+	+	+			+
2,4-Dimethylhexane										+				
2,2-Dimethyl-pentane													+	
Ethanol	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ethyl acetate	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Isopropanol	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hexanal					+						+	+		
Hexane	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Methanol	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Methacrolein						+	+				+	+		
Methylcyclo-pentane	+	+	+	+	+					+	+		+	+
2-Methylfuran										+				
2-Methyl-pentane	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3-Methyl- pentane	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2-Methyl-1-propene	+	+	+	+	+			+	+	+	+	+	+	
2-Methyl-propanal										+		+		
2-Methyl-2-propanol							+	+	+	+				
1-Methoxy-2-propanol										+				
Methylvinyl-ketone							+	+	+	+	+	+		
Pentanal										+	+	+		+
Pentane	+	+	+	+	+	+		+	+	+	+	+	+	+
Propanal						+	+			+	+	+		
2-Propenal	+					+	+			+	+	+		
2,4,4-Trimethyl-1-pentene											+	+		
Trichloro-methane		+	+	+	+	+	+	+	+	+			+	+
Trimethylsilanol								+	+	+				
THF		+	+	+	+		+	+	+	+			+	
Toluene	+	+	+	+	+	+	+	+	+	+	+	+	+	+

The VOCs concentrations in two samples of breath that were measured in two different fashions were compared. The first one was manually SPME extracted directly from the bag containing the breath. In the second case, the sample was transferred into an HS vial and measured. Figure 1 shows the two overlapped chromatograms of exhaled air. The samples measured in the vials display an elevated concentration of the

selected compounds (e.g., butane, pentane, 2-methylpentane, 3-methylpentane, methylcyclopentane, hexane, and toluene) and false positive results for the other. Table IV presents data obtained for in bag and in vial sampling. In the first case, the extraction process was performed in a Tedlar bag. In the second, the sampling was done in vials, using septum brand 1 (butyl-PTFE).

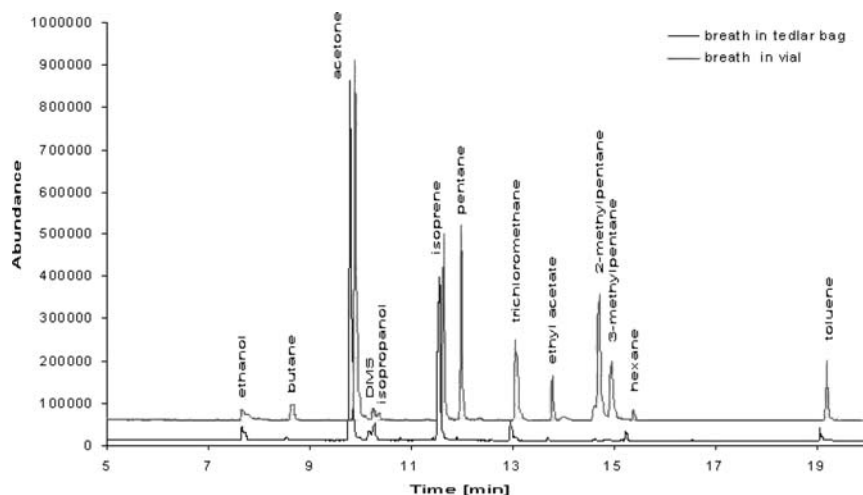


Figure 1. The overlapped SPME–GC–MS chromatograms of exhaled air: (top chromatogram) the extraction process was performed in Tedlar bag, (bottom chromatogram) the same breath but the extraction was done in vial crimped with septum brand 1.

Table IV

Concentrations of Selected VOCs Identified in Breath. Preconcentration was Performed in a Tedlar bag and in the Vial with Septum Brand 1

Compounds	Breath analysis from Tedlar bag (ppb)	Breath analysis from vial with septum (ppb)
Acetaldehyde	41	38
Ethanol	209	259
Butane	19	45
Acetone	746	871
Isoprene	198	196
Pentane	7	18
2-Methylpentane	16	34
3-Methylpentane	17	35
Hexane	37	69
Toluene	142	462

A tree model, performed in Statistica 7.1 Data Miner (Statsoft, Polska) software running on a Windows XP platform, showed that septa brand 1–5 and 13 reveal similar off-gassing products. Additionally, these septa are characterized by a low emission of VOCs. The received dendrogram indicates also a high similarity of VOCs emitted for the membranes brand 11, 12, and 6, 7. A cluster analysis confirmed that septa brand 9 and 7 are quite different.

Time-dependant emission has been investigated for septum butyl–PTFE (brand 1), and the results are presented in Figure 2. The first sample was measured 5 min after crimping. The second vial was used 1 h later, and the last three were sequentially injected in 1 h intervals. Each sample was equilibrated during 5 min and 1, 2, 3, and 4 h respectively, prior to analysis. The compounds, such as acetone and pentane, were on a similar level over a 4 h period. Only the concentration of methylcyclopentane, ethyl acetate, and 3-methylpentane slightly increased. Decreasing concentrations of hexane between time point 1 and 2, or toluene between time points

1–3, might be a result from a poorly established physico-chemical equilibrium in the vial. In the case of most determined substances, after 1 h, the concentration of the released VOCs was stable.

Baking septa in an incubator under a vacuum was tried in order to eliminate the VOCs emission. However, heating the septa at 90°C for 30 days (without opening the incubator) did not decrease off-gassing. In addition, an increasing emission (20–25%) of carbon disulfide for butyl rubber septa was observed.

In view of these results, the application of such septa is limited to brand 7. In the case of the remaining brands, even a short period of storage gives a remarkable contamination of the samples. Additionally, if the outgassing level is similar to the concentration in the breath, the common correction for the background concentrations by subtracting it from the exhaled breath concentrations is not suitable.

Conclusions

The selection of a proper, low emission septa for the crimping of HS vials containing breath samples is often essential for a high-throughput SPME–GC–MS analyses. A chromatographic technique, making a blank of pure nitrogen and a control of the laboratory air, allowed for the determination of a group of compounds emitted by commonly applied septa. In the emission from the septa, 39 substances were identified, mainly hydrocarbons. In many cases, volatiles released by membranes are significantly higher than a typical concentration of selected VOCs in a breath. Additionally, outgassing products might interfere with some compounds.

To eliminate this problem, septum baking was not appropriate. Septum made from butyl rubber with a PTFE covering and heated over a 30 day period did not decrease the emission.

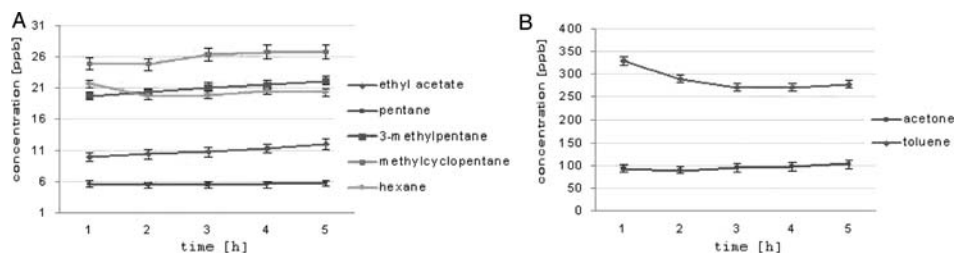


Figure 2. Concentration change of selected VOCs emitted by septum within 4 h. First measurement was done 5 min after crimping, second one, an h after closing, etc.

Therefore, carefully checking every kind of septa, as well as a new batch of received, up to now is suitable for control of emission. However, high quality septa without any emission are still desirable.

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