

Stability of selected volatile breath constituents in Tedlar, Kynar and Flexfilm sampling bags

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Abstract

Stability of 41 selected breath constituents in three types of polymer sampling bags, Tedlar, Kynar, and Flexfilm, was investigated using solid phase microextraction and gas chromatography mass spectrometry. The tested molecular species belong to different chemical classes (hydrocarbons, ketones, aldehydes, aromatics, sulphurs, esters, terpenes, etc.) and exhibit close-to-breath low ppb levels (3-12 ppb) with the exception of isoprene, acetone and acetonitrile (106 ppb, 760 ppb, 42 ppb respectively) Stability tests comprised the background emission of contaminants, recovery from dry samples, recovery from humid samples (RH 80% at 37°C), influence of the bag's filling degree, and reusability. Findings yield evidence of the superiority of Tedlar bags over remaining polymers in terms of background emission, species stability (up to 7 days for dry samples) and reusability. Recoveries of species under study suffered from the presence of high amounts of water (losses up to 10%). However, only heavier volatiles, with molecular masses higher than 90, exhibited more pronounced losses (20-40%). The sample size (the degree of bag filling) was found to be one of the most important factors affecting the sample integrity. To sum up, it is recommended to store breath samples in pre-conditioned Tedlar bags up to 6 hours at maximum possible filling volume. Amongst remaining films, Kynar can be considered as an alternative to Tedlar; however, higher losses of compounds should be expected even within the first hours of storage. Due to the high background emission Flexfilm is not suitable for sampling and storage of samples for analyses aiming at volatiles at low ppb level.

Keywords:

Breath VOCs, stability, recovery, sampling bags, Tedlar, Kynar, Flexfilm, sample storage, SPME-GCMS

1. Introduction

Preservation of the sample integrity during sampling and sample storage is probably one of the most demanding challenges in analytical chemistry. Different phenomena accompanying these phases of analysis like, e.g., background emission of pollutants, losses and interactions between sample constituents irreversibly modify the original sample composition and consequently distort the final results of analyses. This is particularly true in case of exhaled breath analysis. Ultra low concentrations of volatile organic breath constituents (from low ppb to low ppt), presence of highly reactive species and high humidity inducing wet chemistry make breath samples particularly vulnerable to all problems related to storage {Amann, 2011 #1; Amann, 2005 #4; Bajtarevic, 2009 #2; Miekisch, 2004 #6; Phillips, 2008 #7; Poli, 2005 #8}. Despite availability of real-time techniques such as proton transfer reaction mass spectrometry (PTR-MS) {King, 2012 #25; King, 2012 #27; Koc, 2011 #28; King, 2011 #29; King, 2010 #30; King, 2009 #9; King, 2010 #10}, or selected ion flow tube mass spectrometry (SIFT-MS) {Endre, 2011 #11; Spanel, 2011 #12}, gas chromatography–mass spectrometry (GC-MS) remains the gold standard for the analysis of breath constituents {Amann, 2011 #1; Amann, 2005 #4; Bajtarevic, 2009 #2; Ligor, 2009 #32; Phillips, 2008 #7}. Since GC-MS analysis of exhaled air is usually coupled with a time-consuming sample pre-concentration method (e.g., solid phase microextraction (SPME), sorbent trapping, or needle traps (NTD)), sample storage is an inherent part of the analytical procedure. In this context the selection of the optimal storage conditions for breath samples is of particular importance.

Currently, Tedlar (PVF polyvinyl fluoride) is one of the most popular and commonly accepted materials for collecting gaseous samples in general and breath gas samples in particular {Amann, 2011 #1; Bajtarevic, 2009 #2; Buszewski, 2008 #5; Beauchamp, 2008 #13; Steeghs, 2007 #14; Mochalski, 2009 #15; Ligor, 2008 #16; Kushch, 2008 #18; Schwarz, 2009 #19; Schwarz, 2009 #33; Erhart, 2009 #34}. This is due to its moderate price, inertness, relatively good durability, and reusability. Nevertheless, recent access limitations for this type of polymer film caused by the policy of the polymer manufacturer (DuPont, USA) have generated demands for alternative materials suitable to replace Tedlar in breath gas analysis.

A number of studies have investigated the storage of breath constituents in polymer bags. Nevertheless, the majority of studies focused on a limited number of species at levels much higher than the ones observed in breath, or dealt with a single analyte related to a specific disease or disorder {Beauchamp, 2008 #13; Steeghs, 2007 #14; Mochalski, 2009 #15; Groves, 1996 #17} {Gilchrist, 2012 #36}. For example, Groves and Zellers {Groves, 1996 #17} tested the influence of high humidity on the recovery of 6 breath-related compounds at the ppm level. The observed differences between dry and wet matrices were smaller than 10%. Steeghs *et al.* {Steeghs, 2007 #14} investigated the stabilities of 7 species (methanol, acetaldehyde, acetone, isoprene, benzene, toluene and styrene) at approximately 100 ppb level over the period of 72 hours in black-layered Tedlar. The results evidenced good recovery (better than 80%) of acetone, isoprene, acetaldehyde and benzene over this storage period. A more detailed study involving 12 breath species at levels of 70-85 ppb was performed by Beauchamp *et al.* {Beauchamp, 2008 #13}. After 10 h of storage, the observed losses were smaller than 20%.

The main goals of this study were the investigation and comparison of stabilities of selected breath constituents in three types of polymers bags, Tedlar, Kynar and Flexfilm, and secondly the identification of optimal storage conditions for breath samples. The 41 selected C3-C10 species represented different chemical classes (hydrocarbons, ketones, aldehydes, aromatics, sulphurs, esters, terpenes, heterocyclic, etc.) and exhibited close-to-breath low ppb concentrations. The tests comprised the background emission of pollutants, recovery from dry and humid (RH 80% at 37°C) samples over the period of 7 days, and the influence of sample size (degree of bag filling) on sample stability. Finally, the effectiveness of the cleaning protocol was examined as a crucial factor for bag reusability. Gas chromatography with mass spectrometric detection coupled with solid phase microextraction (SPME) as the pre-concentration method was selected as the analytical tool during all experiments. In the framework of the present study SPME provides numerous advantages like ease of operation, good sensitivity, excellent reproducibility, full automation and relatively small extraction dependence on humidity {Pawliszyn, 1997 #35}. Finally, this pre-concentration method requires relatively small amount of sample volume (10-20 ml) to perform extraction. The latter feature was particularly beneficial during this study, as the initial volumes of the samples in the bags remained almost unaffected during experiments.

2. Experimental

2.1 Sampling bags

Within this study, three types of sampling bags were compared with respect to the stability of breath constituents:

- 3-L in volume transparent Tedlar (PVF - polyvinyl fluoride) bags (SKC Inc., USA) equipped with a single polypropylene valve (dimensions when deflated: 26 cm × 24.5 cm, film thickness: 50 µm).
- 3-L in volume SamplePro Flexfilm bags (unknown polymer - trade secret of SKC Inc., USA) equipped with a single polypropylene valve (dimensions when deflated: 21 cm × 41.5 cm, film thickness: 76 µm).
- 3-L in volume Kynar (PVDF - polyvinylidene difluoride) bags (SKC Inc., USA) equipped with a single polypropylene valve (dimensions when deflated: 26 cm × 28.5 cm, film thickness: 50.8 µm).

All bags were new and flushed five times with high-purity nitrogen (type 6.0 – 99.9999%) directly before their use.

2.2 Chemicals and standards

Multi-compound test gas mixtures as well as calibration mixtures were prepared from pure liquid or gaseous substances. The majority of them were purchased from Sigma-Aldrich (Vienna, Austria); n-butane (99%), n-pentane (99.8%), n-hexane (99%), n-octane (99.8%), n-decane (99%), isobutane (99%), 3-methyl pentane (99%), 2-butene E and Z (99%), 2-pentene E and Z (99%), 1-hexene (97%), methylcyclopentane (97%), α -pinene (98%), (+)-3-carene (98.5%), p-cymene (99%), D-limonene (99%), eucalyptol (99%), benzene (99.8%), toluene (99.8%), p-xylene (99%), o-xylene (99%), acetone (99.8%), 2-butanone (99.5%), 2-pentanone (99%), 4-heptanone (97%), 2-butenone (99%), propanal (97%), 2-methyl propanal (99.5%), butanal (99%), hexanal (98%), octanal (99%), 2-methyl 2-propenal (95%), furan (99%), 2-methyl furan (99%), 2,5-dimethyl furan (99%), thiophene (99%), 3-methyl thiophene (98%), methyl acetate (99.5%), ethyl acetate (99.9%), n-propyl acetate (98%), methyl methacrylate

(99%), dimethyl selenide (99%), ethyl ether (99.7%), pyrimidine (99%) and acetonitrile (99.8%). Moreover, 2-methyl pentane (99.5%), 4-methyl heptane (97%), isoprene (99%), ethylbenzene (99.8%), dimethyl sulfide (99%), 2-methyl 1-pentene (99.5%) and n-butyl acetate (99.7%) were obtained from Fluka (Switzerland), whereas, 2,4-dimethyl heptane (95%), 2,4-dimethyl 1-heptene (94%) and 4-methyl octane (97.5%) were provided by Chemsampco (USA). 3-methyl furan (98%) was purchased from Acros Organic (Belgium) and methyl propyl sulfide (98%) from SAFC (USA).

The standard mixtures were prepared in two steps. Firstly, multi-compound primary standards were prepared in 1 L glass bulbs (Supelco, Canada). Prior to the use, each bulb was thoroughly cleaned with methanol and dried at 70°C for at least 12 h. Then, the bulb was evacuated using a vacuum membrane pump and approximately 1 µL of liquid (or 0.5 mL of gaseous) analyte was injected through a rubber septum. Next, the bulb was heated to 60°C for 30 min to ensure complete evaporation and subsequently balanced to ambient pressure with high-purity nitrogen (6.0 - 99.9999%). The final calibration or test mixtures were prepared by transferring appropriate volumes of primary standard with Hamilton syringes into sampling bags filled in advance with predefined amounts of high-purity nitrogen. Calibration curves were obtained on the basis of triplicate analyses of 7 mixtures. Humid test mixtures were prepared in an analogous way as dry samples; however, during the last step polymer bags were filled with humid zero-air produced by means of a generator GasLab (Breitfuss Messtechnik, Germany). The GasLab unit comprises an integrated zero-air generator and a humidification module enabling the preparation of gas mixtures at predefined humidity levels. To avoid water condensation and to mimic conditions during breath sampling, the transfer line and polymer bags were maintained at 40°C during the filling procedure.

The great majority of human breath constituents exhibit very low concentration levels ranging from ppt to several ppb {Amann, 2005 #4; Bajtarevic, 2009 #2; Miekisch, 2004 #6; Ligor, 2008 #16; Amann, 2011 #1}. Consequently, an effort has been made to investigate stabilities of breath compounds at levels close to the ones observed in real samples. Effectively, the multi-compound test mixture contained analytes with concentrations falling within the range of 3-12 ppb. The three exceptions were acetone (720 ppb) and isoprene (106 ppb) exhibiting higher physiological levels in human breath {Kushch, 2008 #18; Schwarz, 2009 #19}, as well as acetonitrile (42 ppb) showing higher LOD for the applied analytical method. The range of volume fractions used during calibration and validation of the analytical method as well as the compounds' concentration levels in the multi-compound test mixture are presented in Table 1.

2.3 SPME procedure and chromatographic analysis

The test gas samples were taken using a 20 ml gas-tight glass syringe (Roth, Germany) equipped with a replaceable needle. Sampling was achieved manually by drawing a volume of 18 ml from the sampling bag and subsequent injection of this volume into an evacuated SPME vial (20 ml in volume, Gerstel, Germany) sealed with a 1.3 mm butyl/PTFE septum (Macherey-Nagel, Germany). To avoid losses of analytes during the sample storage in SPME vials an effort was made to analyze samples within 3 h after the vial filling. The SPME procedure was carried

out automatically using a multipurpose sampler MPS (Gerstel, Germany). SPME was achieved by inserting a 75 μm carboxen-polydimethylsiloxane (CAR-PDMS) fiber (Supelco, Canada) into the vial and exposing it to its content for 10 minutes at 37°C. Immediately after extraction, the fiber was introduced into the inlet of the gas chromatograph where the sorbed VOCs were thermally desorbed at 290°C. The fiber was conditioned at 290°C for 5 minutes prior to each analysis.

The GC-MS analyses were performed using an Agilent 7890A/5975C GC-MS system (Agilent, USA). During the fiber desorption, the split/splitless inlet operated in a splitless mode (1 min), followed by the split mode at ratio 1:20. The analytes under study were separated using a PoraBond Q column (25 m x 0.32 mm, film thickness 5 μm , Varian, USA) working in a constant flow mode of helium at 1.4 ml/min. The column temperature program was as follows: 40°C for 2 min, increase to 260°C at a rate of 7°C/min, held at 260°C for 7 min. The mass spectrometer worked in a SCAN mode with an associated m/z range set from 20 to 200. The quadrupole, ion source and transfer line were kept at 150°C, 230°C and 280°C, respectively.

The identification of compounds was performed in two steps. First, the peak spectrum was checked against the NIST mass spectral library. Next, the NIST identification was confirmed by the retention times obtained on the basis of standards prepared from pure compounds. The retention times of the investigated compounds for the applied chromatographic parameters are presented in Table 1.

2.4 Sampling bags tests

2.4.1 Background test

To identify contaminants emitted by the polymer films 3 new bags of each type were filled with 2000 ml of high-purity nitrogen (corresponding to approximately 67% of their nominal volume), stored at room temperature for 24 hours and analyzed after certain time periods. The time intervals of the measurements were defined as follows: the first sampling was carried out immediately after filling the bag, subsequent ones after 6, 12 and 24 h of storage. Additionally, blank (nitrogen) and laboratory air measurements were performed.

Next, an additional cleaning procedure was applied to check if it is possible to reduce contaminant emission from the bags under study. For this purpose, after five-fold flushing bags were filled with nitrogen and heated overnight (approximately 12 h) in the oven at 50°C to induce potential contaminant desorption from the polymer film or from the valves. Next, bags were again flushed five times with nitrogen and the aforementioned background test procedure was repeated, however, in this case only 3 samplings were performed; immediately after filling, and after 6 and 24 hours of storage.

2.4.2 Dry standard stability test

To investigate the stability of breath species a 41-component test mixture was prepared using the aforementioned procedure and introduced into the tested polymer bags. The nominal levels of all compounds in the test mixture are presented in the Table 1. To study the influence of different (film) surface-to-(sample)volume ratios (SA:V) on the sample integrity three bags of each type of film were filled with different volumes of standard mixture; 2.4, 1.2 and 0.6 L (i.e., 80, 40, and 20 % of the maximum capacity). Due to some differences in the film dimensions these

volumes corresponded to SA:V ratio values of 53, 106, and 212 m⁻¹ for Tedlar bags, 73, 145, and 291 m⁻¹ for Flexfilm bags, and 62, 124, 247 m⁻¹ for Kynar bags respectively. All bags were filled at the same time with the same test mixture and were stored at room temperature (24°C) exposed to daylight. The stability of the test gas was monitored over a period of 7 days with the time instants for drawing the samples defined as follows: the first sample was taken approximately 10 minutes after the bag filling, next ones after 6, 24, 48, 72, 126 and 168 hours of storage.

To confirm repeatability, the stability test was repeated for the test gas mixture volume of 2.4 L with the same sampling protocol, however, with three bags of each type being involved.

2.4.3 Humid standard stability test

Stability of compounds under study in humid matrices was investigated using a test mixture having similar water content as breath, i.e., RH of 80% at 37°C. Three new bags from each type of film were filled with 2.4 L of humid test mixture and sampled immediately after filling and after 6, 24 and 48 h of storage. To avoid condensation and to mimic the sampling of real breath samples (having body temperature) during filling all bags were heated to 37°C. However, during experiment they were stored at room temperature. The duration of the experiment was restricted to 2 days as water vapor permeates relatively easily through all tested polymer films and after a few hours sample humidity reaches ambient levels {Beauchamp, 2008 #13; Cariou, 2006 #20; Beghi, 2006 #21}. Additionally, one bag of each type was sampled after 2 and 4 hours to study the evolution of the VOCs concentrations during the first hours of storage, when the humidity still remains elevated.

2.4.4 Reusability test

The reusability test was focused on studying the effectiveness of the bag cleaning protocol developed during one of our previous studies {Mochalski, 2009 #15}. Polymer bags involved in the dry standard stability test (i.e., containing test mixture for 7 days) were used during the test. Firstly, bags were flushed five times with high purity nitrogen to remove remainings of the test gas. Next, all bags were filled with 2 L of nitrogen and conditioned at 50°C for approximately 12h to remove volatiles of interest from the bags' material (film, valve etc.). After the heating, bags were again rinsed five times, filled with 2 L of high-purity nitrogen and stored at room temperature for 24 hours. The effectiveness of the applied cleaning protocol was checked by comparing the levels of test mixture VOCs before and after this time of storage.

3. Results and discussion

3.1 Method validation

Limits of detection (LODs) were calculated using the mean value of the blank responses and their standard deviations obtained on the basis of 10 blank measurements {Huber, 2003 #22} and are presented in Table 1. The limit of quantification (LOQ) was defined as three times the LOD. The relative standard deviations (RSDs) were calculated on the basis of five consecutive analyses of standard mixtures. The calculated RSDs varied from 1-9% and were recognised as satisfactory for the aims of this study. The system response was found to be linear within the investigated concentration ranges, as shown in Table 1, with coefficients of variation ranging from 0.954 to 0.999.

3.2 Background test

All volatiles found to be emitted by the investigated polymer sampling bags are summarised in Tables 2-4. The presented concentrations are the mean values of VOCs levels in three bags. The emission rates were calculated for unconditioned bags assuming that the contaminants are emitted by polymer film.

A total of 27 compounds were emitted by Flexfilm bags. The most dominant chemical classes were hydrocarbons with 14 and aldehydes with four species, respectively. Amongst the remaining compounds, there were three ketones, two esters, two volatile sulphur compounds, one aromatic and one amide. Sulphur compounds (COS and CS₂) were found to be produced by all three types of bags and seem to be emitted by rubber parts of the sampling valves {Mochalski, 2009 #15}. As the emission of these two species was investigated thoroughly in our previous paper {Mochalski, 2009 #15} they were not quantified within this study. Acetone was the most abundant compound with the concentration level reaching 140 ppb after 24 hours of storage. Apart from acetone, high concentrations were observed for some hydrocarbons (2-butene, n-butane, methylcyclopentane, n-hexane, 2,4-dimethyl heptane). 75% of the contaminants were detected shortly after filling the Flexfilm bag. Six hours later all of them were present in the bags at levels of several ppb. In the context of breath gas analysis this emission can be considered as significant. Pre-conditioning of Flexfilm bags reduced the emission of aldehydes, ketones and esters by 50-80%, however, the emission rates of hydrocarbons remained intact (with the exception of 2-butene and toluene). Acetone background was particularly improved with levels spreading around 20 ppb after conditioning and 1 day of storage. Nevertheless, despite conditioning considerable concentrations of contaminants could be found after several hours of storage. Repeating the pre-conditioning step (data not shown) further improved the background of all species apart from hydrocarbons.

Kynar bags released 21 species. The predominant chemical class were aromatics with four compounds. Apart from them, there were two hydrocarbons, two volatile sulphur compounds (COS and CS₂), three aldehydes, three ketones and three esters, one CFC, one halide and one nitro compound. In case of unconditioned Kynar bags the great majority of contaminants could be detected in small amounts (usually below 1 ppb) after 6 hours of storage. At the end of the experiment the highest levels were noted for acetone and toluene; 33 and 9.4 ppb respectively. The applied pre-conditioning method was found to be very efficient in case of Kynar bags. After cleaning only five species (2 butanone, toluene, p-xylene, COS and CS₂) could be detected in the Kynar bag samples after 6 hours of storage. After 1 day several additional contaminants were found in the Kynar bags in detectable amounts, however, their levels were below the LOQs of the analytical method. Taking into account the good results of the pre-conditioning protocol it can be surmised that additional cleaning/s could further reduce the contaminants emission to the levels acceptable for breath analysis. Conversely, conditioning promoted the emission of sulphur species – COS and CS₂ – which is consistent with the findings of our previous paper {Mochalski, 2009 #15} indicating rubber parts of polymer bags (o-ring, septum) as potential sources of these species.

Only 9 compounds were found to be emitted by Tedlar film: three hydrocarbons (n-hexane, 2,4 dimethylheptane and 4 methyl octane), two volatile sulphur compounds (COS and CS₂), N,N-dimethylacetamide, phenol, acetonitrile and 1-methoxy-2-propyl acetate. N,N-dimethylacetamide and phenol are commonly known and well documented contaminants in Tedlar bags {Beauchamp, 2008 #13; Trabue, 2006 #23}. Amongst the quantified species the highest levels were noted for acetonitrile (19 ppb). 2,4 dimethylheptane and 4 methyl octane could be detected within few hours of storage, however, n-hexane was found only at the end of experiment. Like in case of Kynar bags pre-conditioning considerably improved the background emission. However, small amounts of 2,4 dimethylheptane and 4 methyl octane could still be detected after 6 hours of storage.

Several compounds identified as contaminants in tested bags were found also in room air at the low ppb level inducing their permeation from the room air as the possible source of pollution (acetone, 2 butanone, n-butane, n-pentane, methyl acetate, toluene, p-xylene). Nevertheless, the absence in the bags content other detected in room air species, having similar physicochemical properties favours the emissions from the polymer film as the main source of contamination. For example, 2 methyl butane – hydrocarbon very similar to n-pentane - present in room air at the level of several ppb was not detected in the bags content during the background test.

3.3 Dry standard stability test.

The stability of test mixture compounds in tested polymer bags over the period of one week is presented in Table 5. The compound's concentration was considered stable when its level was higher than 80% of its initial value.

For all bags the stability of compounds of interest was strongly correlated with the volume of the test sample filled into the bag. Regardless of the chemical class of a compound, its recovery was significantly better when sampling bag was filled up to 80% of its nominal volume. For the majority of all species, SA:V ratios below 100 m⁻¹ provided good recoveries even after 7 days of storage. Samples with the highest surface-to-volume ratios (above 200 m⁻¹) were stable only for several hours. This finding is not surprising, as the area of the bag materials (polymer film, valve, etc) having contact with the sample for all SA:V ratios remained the same and its potential for interactions with the sample constituents was comparable. Consequently, large samples containing higher masses of the investigated species were more resistant to losses during storage. Additionally, samples stored at lower SA:V ratios were less susceptible to the emission of contaminants, as can be seen in Table 5. For example, in Flexfilm bags after 24 hours of storage the concentration of n-butane remained stable when the bag was filled up to 80% of its nominal volume, whereas in the bags filled up to only 20% of its nominal volume its concentration increased two-fold. Thus, it is strongly recommended to collect the largest possible volume of the sample in order to provide the optimal conditions for the preservation of its integrity.

Considerable differences were found for the stabilities of compounds in different polymer bags. Since the superiority of lower SA:V ratios of stored samples is undeniable further discussion of the compound recoveries will refer to bags filled up to 80% (2.4 L) with test mixture, unless otherwise stated.

Aliphatic Hydrocarbons

The stabilities of hydrocarbons tested within this study suffered significantly from the background emission in Flexfilm and Kynar bags. Despite pre-conditioning, levels of numerous species tended to increase rapidly (even within 6 hours of storage). This phenomenon was particularly pronounced for Flexfilm bags, confirming the finding of the background tests. For HCs found not to be emitted by bags material good recoveries were noted even after 3 days of storage. Due to the much lower background emission Kynar bags provided better stability of HCs. The majority of species from this chemical class remained stable up to 3 days of storage (when filled up to 80% of maximum volume). Nevertheless, the risk of contamination considerably limits the applicability of Kynar and Flexfilm bags during breath studies aiming at hydrocarbons at low ppb levels. Hydrocarbons stored in Tedlar bags exhibited excellent recoveries over the whole investigated storage period, even for higher SA:V ratios. Only heavier hydrocarbons (e.g., n-decane) showed higher losses. Specifically, unsaturated hydrocarbons were much better preserved in Tedlar bags than in other ones. Interestingly, in Kynar and in Flexfilm bags the drop of isoprene levels (initial value of 106 ppb) was accompanied by the increase of 2-methyl 2 propenal, 3-buten-2-one and 3 methylfuran – species known to be the products of the isoprene degradation in the atmosphere {Dibble, 1999 #24}. For example, after 7 days concentrations of these species in Kynar bags filled with 2.4L of test mixture were 12, 11, and 1.8 ppb, respectively. In Tedlar bags this effect was much less evident. In case of this film only 3-buten-2-one was found to be produced (1 ppb after 1 day, 3 ppb after 7 days) Perhaps the presence of Kynar and Flexfilm films promotes the degradation of isoprene. As a result, due to the good background and excellent recoveries Tedlar bags seem to be the best choice for sampling and storage of breath hydrocarbons.

Aromatic hydrocarbons

In Kynar and Flexfilm bags the stability of studied aromatics was relatively poor. In general, acceptable recoveries were observed only up to 24 hours of storage. In samples having higher SA:V ratios losses were pronounced even within the first hours of storage. In Tedlar bags recovery of species from this class was over 80% at the end of investigated period, however, only in bags filled up to 80% of the maximum capacity. In all cases the values of recovery tended to decrease with increasing molecular mass of a compound.

Ketones

Recovery of ketones in Kynar bags was unsatisfactory. Their levels rapidly dropped below the arbitrarily chosen threshold of 80%. Even acetone having an initial concentration of 720 ppb followed this pattern. The apparently better stability of 2-butanone can easily be explained by its background emission from the Kynar film. Consequently, ketones stored in Kynar bags should be analysed within several hours after sampling. Much better recoveries were observed in Flexfilm bags, with characteristic drops related to the molecular mass of the compound. Once more Tedlar bags provided the best storage conditions for discussed species. Apart from 4-heptanone, all ketones were stable for up to 7 days of storage. These results and the fact that Tedlar bags do not exhibit ketone release render this material optimal for the storage of species from this chemical category.

Aldehydes

Flexfilm bags were found to be inappropriate for the storage of aldehydes. Background emission significantly affected their initial concentrations. The 7 day monitoring period even revealed the emission of additional aldehydes (e.g., n-heptanal) not being detected during the 24 hour background test. Nevertheless, n-octanal stored in Flexfilm bags exhibited the best stability. In Kynar and Tedlar bags the stability of all tested aldehydes was comparable (up to 3 days).

For all remaining compounds (e.g., sulphurs, esters, terpenes) the superiority of storage in Tedlar bags is undisputed. For species with molecular mass up to 90 Tedlar bags provided good stability for up to 7 days of storage. Recoveries of heavier species were better than 80% only within 3-4 days. In Kynar and Flexfilm containers losses of these analytes were more evident and usually exceeded 20% in samples stored longer than one day. The stability of pyrimidine, acetonitrile and dimethyl selenide was especially poor. In all cases acetonitrile concentrations rapidly dropped even within the first several hours of storage. This finding is consistent with previous studies evidencing huge losses of this compound during storage due to the permeation through the polymer film {Beauchamp, 2008 #13}. Its slightly better recovery in Tedlar bags can be explained by the compensation of losses due to background emission. A similar progression of stability was noted for pyrimidine with losses being acceptable only for Tedlar bags within 6 hours of storage. Dimethyl selenide showed good recoveries only in Tedlar bags.

3.4 Humid standard stability test

The comparison of recoveries of volatiles for dry and humid test mixtures is presented in Table 6. It must be stressed here that water permeates relatively easily through all tested materials and consequently, sample humidity remains elevated only for several hours of storage {Beauchamp, 2008 #13; Cariou, 2006 #20; Beghi, 2006 #21} and reaches ambient level. The contrary holds true for dry samples that exhibit ambient levels of water vapour after a few hours of storage. Consequently, the humid standard stability test was restricted to a period of 48 h only.

For the majority of compounds the difference between recoveries in dry samples and humid samples was smaller than 10%, which is in good agreement with the results obtained by Groves and Zellers {Groves, 1996 #17}. Nevertheless, species in humid samples exhibited usually slightly poorer stability. In general recovery differences tended to increase with increasing molecular masses of the compounds. For the heaviest species studied within this study (n-decane, eucalyptol, D-limonene, p-cymene, α -pinene) they amounted to 20-40%, thus significantly reducing the safe storage time. Interestingly, in Flexfilm and Kynar bags presence of large amounts of water reduced the emission of contaminants. Most probably water condensing and permeating through polymer films forms a kind of barrier protecting samples from background emission of pollutants. The same water layer seems to induce higher losses of less volatile and more soluble species tending to go into the liquid phase. Consequently, a rapid drop in the concentrations of hydrophilic compounds is observed during the first hours of their storage. Amongst the remaining volatiles acetonitrile was especially sensitive to the presence of water with losses of 30% already after 2 hours of storage. The humidity influence is relatively similar for all bag materials tested. To sum up, high humidity is a

crucial factor considerably reducing safe storage time of breath constituents. Since the recoveries of compounds heavier than 90 drop significantly during the first hours of storage it is recommended to analyse breath samples within six hours of storage.

3.5 Reusability test.

The applied cleaning protocol was found to be efficient. In Tedlar bags after 24 hours of storage of pure nitrogen only 4 compounds from the tested ones were detected: 2-butanone, 3 methylthiophene, hexanal and p-xylene. However, their levels were below the LOQs of the applied method. The same number of species under study was found in Kynar bags: acetonitrile, 3 methylthiophene, n-octane and octanal. Amongst them acetonitrile exhibited quantified levels spreading around 4 ppb. Cleaning of Flexfilm containers was more difficult. Excluding species known to be released, six artifacts from the test mixture were detected in these bags after storage for one day: acetonitrile, acetone, pyrimidine, n-octane, p-xylene, and octanal. Acetone showed an average concentration of 18 ppb, whereas acetonitrile levels reached a mean value of 6 ppb. Additional cleaning cycles might be necessary to further remove remainings of the previous sample.

4. Conclusions.

In general, several valuable pieces of information on the storage of breath gas samples (as well as other samples containing species at the ppb level) in polymer bags can be extracted from the results of this study.

Firstly, the background emission of pollutants is one of the most important factors when selecting the optimal polymer. High contaminants release distorts the original sample composition already during sampling (bag filling). In the context of breath research aiming at VOCs at low ppb or even ppt levels Tedlar bags with only nine identified contaminants seem to be the best choice. However, two-fold pre-conditioning of bags before usage is highly recommended. On the other hand, Kynar and particularly Flexfilm were found to emit numerous pollutants (mainly hydrocarbons) detectable immediately, or after few hours of storage at ppt to ppb levels. Pre-conditioning, even when repeated for several times was not efficient in case of Flexfilm bags, consequently, this type of material is only suitable for studies aiming at much higher levels of VOCs (e.g., at the ppm level). Due to the quite effective cleaning Kynar could be considered as an alternative to Tedlar, however, it must be remembered that even repeated conditioning does not guarantee the reduction of contaminant emission to a safe level. Finally, it must be underlined that within this study due to the chromatographic limitations only C3-C11 contaminants were monitored, thus the emission of heavier pollutants cannot be excluded.

Secondly, in case of all tested materials recovery of volatiles strongly depends on the degree of bag filling (i.e., on the polymer surface-to-(sample)volume ratio (SA:V)). The recoveries of the investigated species in bags with low SA:V values (below 100 m^{-1}) were satisfactory up to 7 days of storage. The increase of the SA:V ratios values above 200 decreases the storage time with acceptable recovery (>80%) by a factor of 3-6. This finding is not surprising as the VOCs levels in smaller samples (containing smaller masses of species) are more vulnerable to losses related to sorption or permeation. Additionally, samples in bags filled up to 80% of their maximum volume

were less affected by the background emission of contaminants. Consequently, if breath samples are to be stored in polymer bags it is strongly recommended to collect a sample volume as large as possible.

Stability comparisons of the analytes under study in the three polymer bags demonstrated the supremacy of Tedlar over remaining films. In the case of a dry test mixture, recoveries from Tedlar bags (when filled up to 80% of nominal volume) were excellent even after one week of storage. Nevertheless, this safe storage time decreases with the increase of the VOC's molecular mass. For Kynar the storage times for which an acceptable stability of the tested VOCs can be expected is generally shorter. In this type of container low ppb VOCs should be analyzed within one day of storage. Nevertheless, Kynar is not suitable for storage of some classes of compounds like ketones (poor recovery), or hydrocarbons (high background emission, or polymer dependent decomposition as it may be the case for isoprene)). The suitability of Flexfilm bags for storing breath C3-C10 species is very limited. Although the losses of compounds under study in this polymer were lower than in Kynar bags, the levels of pollutants (hydrocarbons, aldehydes, ketones) were especially high, thereby considerably affecting sample integrity.

High humidity affects the species' recoveries. For the majority of compounds stabilities in humid air were up to 10% lower than in a dry matrix. Higher losses (20-40%) detectable even shortly after the bags filling were observed for volatiles with molecular mass above 110. Consequently, in order to reduce losses of heavier species it is advised to analyze breath samples within 6 hours after sampling.

All tested polymers can be reused. The applied cleaning protocol was found to be quite efficient for the removal of artifacts from a previous sample. Nonetheless, to provide maximum security the cleaning procedure proposed here should be repeated at least two times.

In the context of reusability one important factor was not investigated within this study, namely the ageing effect of the polymer film. As it was demonstrated in our previous paper {Mochalski, 2009 #15} that used polymer bags with scratched film exhibit poorer recoveries for sulphur compounds. A similar effect is expected for other classes of species. Therefore, an effort must be made to protect the polymer film and control its quality during usage.

Finally, it must be stressed that due to limitations of the analytical method only C3-C11 volatiles were tested within study. For heavier or for more reactive species problems arising from sample storage can be much more apparent and demand additional studies.

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Table 1. Retention times R_t (min), LODs (ppb), RSDs (%), coefficients of variation (R^2), linear ranges (ppb) of compounds under study and levels of species in the multi-compound test mixture. Compounds are ordered with respect to retention time.

VOC	CAS	R_t [min]	Test mixture level [ppb]	RSD [%]	LOD [ppb]	R^2	linear range [ppb]
Isobutane	75-28-5	10.90	6	6.7	0.32	0.998	1-30
2-Butene, (E)	624-64-6	11.01	-	1.8	0.3	0.995	1-17
2-Butene, (Z)	590-18-1	11.11	-	3	0.3	0.994	1-22
Acetonitrile	75-05-8	11.44	42	7.4	4	0.999	12-62
n-Butane	106-97-8	11.85	6.2	4.3	0.19	0.987	0.63-25
Furan	110-00-9	13.39	12	2.1	0.22	0.999	0.6-22
Propanal	123-38-6	13.51	22	2.6	0.6	0.997	2-45
Acetone	67-64-1	13.65	720	2.6	0.74	0.999	3-1000
Dimethyl sulfide	75-18-3	14.33	10	1.5	0.1	0.999	0.3-30
Methyl acetate	79-20-9	15.06	12	2.2	0.14	0.999	0.4-25
Ethyl ether	60-29-7	15.94	8	1.2	0.29	0.999	1-20
Isoprene	78-79-5	16.10	106	1.3	0.1	0.999	0.5-175
2-Pentene, (E)	646-04-8	16.32	8	1.6	0.1	0.999	0.4-10
2-Pentene, (Z)	627-20-3	16.48	5	2.5	0.14	0.998	0.3-6
n-Pentane	109-66-0	16.57	6.2	1.6	0.11	0.996	0.4-25
Dimethyl selenide	593-79-3	16.76	10	3	0.23	0.998	0.6-12.2
2-Propenal, 2-methyl-	78-85-3	16.98	-	1	0.11	0.998	0.4-29
Propanal, 2-methyl-	78-84-2	17.25	-	6.5	0.26	0.997	0.8-15.7
3-Buten-2-one	78-94-4	17.59	-	5	0.19	0.998	0.6-23
Butanal	123-72-8	18.03	-	3	0.4	0.988	1.2-12
Furan, 2-methyl-	534-22-5	18.10	7	2	0.1	0.998	0.3-18
2-Butanone	78-93-3	18.20	9	7	0.13	0.997	0.4-36
Furan, 3-methyl-	930-27-8	18.39	-	3	0.15	0.997	0.4-20
Ethyl Acetate	141-78-6	18.96	8	2.2	0.13	0.996	0.4-17
Thiophene	110-02-1	19.93	9	3.3	0.15	0.999	0.45-21
1-Pentene, 2-methyl-	763-29-1	19.95	-	3.2	0.1	0.999	0.3-15
Pentane, 2-methyl-	107-83-5	20	9.5	1	0.18	0.999	0.55-11
Pentane, 3-methyl-	96-14-0	20.19	-	1.5	0.1	0.999	0.4-12
1-Hexene	592-41-6	20.22	9	1.7	0.2	0.999	0.6-10
Benzene	71-43-2	20.38	12	3.8	0.3	0.998	1-36
Cyclopentane, methyl-	96-37-7	20.45	-	9	0.1	0.991	0.3-11
n-Hexane	110-54-3	20.70	6.2	1.6	0.12	0.995	0.4-25
Pyrimidine	289-95-2	21.70	10	9	0.1	0.972	0.4-28
2-Pentanone	107-87-9	21.98	8	2.2	0.1	0.998	0.4-24
Furan, 2,5-dimethyl-	625-86-5	22.04	7.5	1.4	0.08	0.999	0.3-15
Methyl methacrylate	80-62-6	22.11	-	1.6	0.11	0.999	0.4-18
n-Propyl acetate	109-60-4	22.71	-	1.3	0.15	0.999	0.5-17

Methyl propyl sulfide	3877-15-4	22.73	6	2.1	0.04	0.996	0.2-30
Thiophene, 3-methyl-	616-44-4	24.00	6.5	4.2	0.1	0.996	0.3-22
Toluene	108-88-3	24.30	12	2.9	0.1	0.993	0.3-30
Hexanal	66-25-1	25.76	5	9	0.4	0.996	1.2-10
n-Butyl acetate	123-86-4	26.21	6	2.1	0.4	0.995	1.2-12
Heptane, 4-methyl-	589-53-7	26.76	-	2.8	0.24	0.989	0.6-11.6
Ethylbenzene	100-41-4	27.45	-	7	0.25	0.989	0.75-13
n-Octane	111-65-9	27.60	8	2.8	0.1	0.998	0.3-14
p-Xylene	106-42-3	27.72	8.5	8	0.07	0.986	0.3-18
o-Xylene	95-47-6	28.03	-	6	0.15	0.991	0.4-15
4-Heptanone	123-19-3	28.36	5	6.3	0.06	0.978	0.2-17
Heptane, 2,4-dimethyl-	2213-23-2	28.98	-	5.9	0.1	0.987	0.3-8.8
1-Heptene, 2,4-dimethyl-	19549-87-2	29.05	-	6	0.12	0.986	0.4-9
Octane, 4-methyl-	2216-34-4	29.76	7	3.4	0.2	0.995	0.6-11
α -pinene	80-56-8	30.81	6	8	0.46	0.985	1.4-19
Octanal	124-13-0	31.87	3	11	0.3	0.974	1-17
3-carene	13466-78-9	32.14	4	5.3	0.61	0.954	1.8-12
p-Cymene	99-87-6	32.67	7	5.7	0.1	0.973	0.4-21
D-Limonene	5989-27-5	32.88	9	6	0.45	0.954	1.4-18
n-Decane	124-18-5	33.21	7	9	0.4	0.978	1.2-17
Eucaliptol	470-82-6	33.46	8.5	6.1	1	0.986	3-25
n-Dodecane	112-40-3	36.22	-	8	0.5	0.964	1.5-15

Table 2. Contaminants emitted by Flexfilm bags. Compounds are ordered with respect to the increasing retention time. “-” denotes that the VOC was not detected, whereas “<LOQ” stands for VOC level below LOQ.

VOC	CAS	New bag sampling time [h]				Preconditioned bag sampling time [h]			VOC emission $\times 10^{-12}$ [g \times h $^{-1}$ \times cm $^{-2}$]
		0	6	12	24	0	6	24	
Carbonyl sulfide (COS)	463-58-1	Not quantified							
Acetaldehyde	75-07-0	Not quantified							
2-Butene, (E)	624-64-6	-	1.6	2.0	2.3	-	-	0.6	0.39
2-Butene, (Z)	590-18-1	-	3.6	6.7	12.2	-	0.8	3.6	1.53
n-Butane	106-97-8	1.6	6.2	11.1	19.1	<LOQ	3.7	8.1	2.62
Propanal	123-38-6	<LOQ	1.4	2.1	2.4	-	-	-	0.48
Acetone	67-64-1	14	75	102	140	-	9.9	19	25.7
Carbon disulfide (CS ₂)	75-15-0	Not quantified							
Methyl acetate	79-20-9	<LOQ	<LOQ	0.3	0.4	-	-	-	0.1
n-Pentane	109-66-0	0.4	2.0	3.4	5.5	0.4	1.7	3.4	0.99
2-Propenal, 2-methyl-	78-85-3	<LOQ	<LOQ	0.3	0.4	-	-	<LOQ	0.1
3-Buten-2-one	78-94-4	<LOQ	<LOQ	0.6	0.6	-	<LOQ	<LOQ	0.19
Butanal	123-72-8	<LOQ	2.2	3.6	4.5	-	1.3	1.3	1.01
2-Butanone	78-93-3	0.5	1.8	2.9	4.1	-	0.6	1.0	0.85
Pentane, 2-methyl-	107-83-5	<LOQ	0.7	1.1	1.8	<LOQ	1.4	3.0	0.41
1-Hexene	592-41-6	<LOQ	0.9	1.4	2.2	<LOQ	0.8	1.6	0.49
Pentane, 3-methyl-	96-14-0	<LOQ	1.1	2.0	3.1	0.4	1.7	3.8	0.68
Cyclopentane, methyl-	96-37-7	1.7	6.6	11.1	17.3	2.0	9.2	18.7	3.78
n-Hexane	110-54-3	3.8	17.1	28.6	44.8	4.8	22.7	45.6	10

Methyl methacrylate	80-62-6	1.6	6.7	10.1	14.6	0.4	1.4	2.6	4.22
Toluene	108-88-3	<LOQ	1.2	1.8	2.4	<LOQ	0.3	0.5	0.67
Heptane, 4-methyl-	589-53-7	-	<LOQ	1.2	2.0	-	1.2	2.4	0.53
Heptane, 2,4-dimethyl-	2213-23-2	1.9	8.9	14.8	23.2	3.4	14.8	30.4	7.76
2,4-Dimethyl-1-heptene	19549-87-2	-	<LOQ	0.6	0.8	<LOQ	0.5	1.1	0.26
Octane, 4-methyl-	2216-34-4	<LOQ	2.8	4.5	6.8	1.0	4.1	8.6	2.37
Caprolactam	105-60-2	Not quantified							
Dodecane	112-40-3	<LOQ	3.3	5.3	6.4	2.0	3.3	5.2	3.51

Table 3. Contaminants emitted by Kynar bags. Compounds are ordered with respect to the increasing retention time. “-” denotes that the VOC was not detected, whereas “<LOQ” stands for VOC level below LOQ.

VOC	CAS	New bag sampling time [h]				Pre-conditioned bag sampling time [h]			VOC emission $\times 10^{-12}$ [g \times h $^{-1}$ \times cm $^{-2}$]
		0	6	12	24	0	6	24	
Carbonyl sulfide (COS)	463-58-1	Not quantified							
Acetone	67-64-1	2.7	41.4	29.4	32.8	-	-	-	11
Carbon disulfide (CS ₂)	75-15-0	Not quantified							
Trimethylsilyl fluoride	420-56-4	Not quantified							
Methyl acetate	79-20-9	-	<LOQ	0.4	0.5	-	-	-	0.14
n-Pentane	109-66-0	-	-	-	0.5	-	-	<LOQ	0.08
2-Propenal, 2-methyl-	78-85-3	-	<LOQ	0.4	0.4	-	-	-	0.12
Propanal, 2-methyl-	78-84-2	<LOQ	1.3	1.6	1.9	-	-	<LOQ	0.59
2-Butanone	78-93-3	0.5	2.3	3.6	4.3	<LOQ	0.5	0.7	1.19
Ethyl Acetate	141-78-6	<LOQ	1.0	1.4	1.8	-	-	<LOQ	0.59
1,2-Dichlorohexafluoropropane	661-97-2	Not quantified							
n-Hexane	110-54-3	-	<LOQ	<LOQ	0.4	-	-	<LOQ	0.12
Propane, 2-nitro-	79-46-9	Not quantified							
2-pentanone	107-87-9	-	<LOQ	<LOQ	<LOQ	-	-	-	0.09
n-Propyl acetate	109-60-4	<LOQ	0.6	0.8	1.0	-	-	-	0.43
Toluene	108-88-3	1.2	5.6	7.4	9.4	-	0.3	0.4	3.44
Hexanal	66-25-1	-	-	1.1	1.1	-	-	-	0.34
1,3-Dioxane, 4,4-dimethyl-	766-15-4	Not quantified							

Ethylbenzene	100-41-4	-	<LOQ	1.0	1.4	-	-	-	0.51
p-Xylene	106-42-3	-	0.5	1.0	1.5	-	0.3	0.4	0.48
o-Xylene	95-47-6	-	<LOQ	0.4	0.5	-	-	-	0.22

Table 4. Contaminants emitted by Tedlar bags. Compounds are ordered with respect to the increasing retention time. “-” denotes that the VOC was not detected, whereas “<LOQ” stands for VOC level below LOQ.

VOC	CAS	New bag sampling time [h]				Preconditioned bag sampling time [h]			VOC emission $\times 10^{-12}$ [g \times h $^{-1}$ \times cm $^{-2}$]
		0	6	12	24	0	6	24	
Carbonyl sulfide (COS)	463-58-1	Not quantified							
Acetonitrile	75-05-8	<LOQ	14.2	17.3	18.9	-	-	-	4.23
Carbon disulfide (CS ₂)	75-15-0	Not quantified							
n-Hexane	110-54-3	-	-	-	0.5	-	-	-	0.15
Acetamide, N,N-dimethyl-	127-19-5	Not quantified							
1-Methoxy-2-propyl acetate	108-65-6	Not quantified							
Heptane, 2,4-dimethyl-	2213-23-2	-	0.5	0.8	1.3	-	0.3	0.4	0.5
4-me-Octane	2216-34-4	<LOQ	0.8	1.0	1.5	-	<LOQ	<LOQ	0.82
Phenol	108-95-2	Not quantified							

Table 5. Stability of selected breath constituents in Tedlar, Kynar and Flexfilm sampling bags for dry matrix. V_f – filling volume in %. The recoveries exceeding 100% reflect the emission of pollutants.

VOC	V_f [%]	Recovery in Flexfilm bag [%]						Recovery in Kynar bag [%]						Recovery in Tedlar bag [%]					
		6 h	24 h	48 h	72 h	126 h	168 h	6 h	24 h	48 h	72 h	126 h	168 h	6 h	24 h	48 h	72 h	126 h	168 h
n-Butane	20	180	424	714	1020	1621	1978		113	107	109	131	130	95	94	92	90	88	85
	40	159	296	454	607	963	0	93	94	89	85	86	84	93	95	94	91	94	81
	80	142	238	352	471	693	840	112	113	103	106	104	104	104	103	97	93	94	94
n-Pentane	20	120	176	210	296	412	486		94	91	91	84	82	92	93	89	85	79	76
	40	111	132	162	193	260	0	96	92	84	82	78	77	99	93	88	81	85	80
	80	98	113	137	154	170	205	112	110	105	98	96	90	108	105	97	91	91	86
n-Hexane	20	215	472	721	992	1448	1723		103	101	100	129	125	99	97	95	90	82	87
	40	179	347	528	673	1019	0	92	91	88	81	83	75	94	96	90	90	93	82
	80	152	263	387	495	729	855	111	114	107	103	103	107	110	109	99	98	100	96
n-Octane	20	95	89	81	78	69	67		91	86	83	74	75	99	91	89	86	82	78
	40	97	91	85	85	81	0	93	92	88	84	81	78	98	95	88	85	86	80
	80	93	92	89	84	80	80	108	107	102	102	98	84	100	94	93	89	88	84
n-Decane	20	82	71	59	50	56	40		95	78	73	64	57	86	78	69	57	50	50
	40	96	92	69	59	55	0	98	83	78	71	63	49	92	85	76	71	62	57
	80	106	92	83	70	62	56	102	91	94	87	85	76	101	93	86	82	79	73
Isobutane	20	96	88	88	95	96	99		90	93	79	92	86	107	100	86	83	86	84
	40	99	93	86	69	80	0	99	96	79	77	74	80	101	90	88	74	77	74
	80	89	88	69	72	75	74	100	85	85	82	76	89	101	102	85	87	90	83
Pentane, 2-methyl-	20	102	132	138	149	187	203		102	100	89	92	88	94	81	84	83	83	81
	40	91	92	98	95	104	0	101	98	93	84	88	83	89	92	82	85	85	72
	80	87	86	87	85	89	97	110	102	101	95	98	88	110	108	96	98	99	104
Octane, 4-methyl-	20	124	178	231	263	332	358		100	99	104	105	104	105	125	123	147	148	179
	40	110	140	162	166	222	0	98	103	93	105	103	108	91	101	112	110	125	107
	80	110	107	140	131	170	169	99	104	102	103	103	89	106	107	104	108	115	116

2-Pentene, (E)	20	94	93	83	80	82	81		88	75	63	48	37	89	93	88	84	83	81
	40	93	81	85	80	80	0	93	87	73	63	44	30	97	94	90	87	86	82
	80	89	84	87	85	85	82	109	105	97	88	72	58	103	100	93	92	92	82
2-Pentene, (Z)	20	103	94	85	96	82	80		85	77	64	53	41	91	93	90	84	82	80
	40	113	105	104	99	104		93	90	75	66	50	36	96	92	91	85	84	83
	80	94	88	93	88	88	85	109	105	95	89	76	65	106	100	95	95	92	87
1-Hexene	20	96	127	154	172	253	285		99	88	77	78	75	96	92	89	85	83	80
	40	99	112	129	138	173		94	88	85	76	74	68	92	91	84	87	88	80
	80	97	103	111	115	135	145	111	107	101	96	93	88	111	107	95	97	92	96
Isoprene	20	93	87	80	70	70	65		85	77	69	57	47	88	90	86	82	79	77
	40	95	83	83	77	77		93	88	76	71	60	50	97	92	89	86	84	81
	80	91	86	87	83	80	77	109	105	98	92	83	75	110	105	102	99	97	88
Benzene	20	85	80	69	65	58	53		90	77	63	54	54	92	85	79	74	68	65
	40	93	86	82	71	71		88	80	71	65	61	56	88	86	79	77	74	69
	80	93	87	83	73	76	70	105	99	91	84	81	70	108	101	91	87	90	88
Toluene	20	85	78	67	61	56	51		81	66	60	52	46	90	81	73	64	59	55
	40	87	84	79	70	66		88	79	71	63	56	52	90	84	78	72	66	62
	80	92	88	82	77	73	67	100	94	85	78	70	64	104	100	94	90	86	81
p-Xylene	20	80	72	55	49	42	40		71	55	48	40	39	84	69	64	52	45	43
	40	89	78	74	63	63	0	76	69	56	51	48	38	87	79	68	62	59	53
	80	86	83	76	70	64	52	94	84	73	68	58	49	109	105	98	91	87	81
Acetone	20	95	95	92	94	92	90		71	58	53	39	37	89	79	71	65	55	52
	40	90	93	90	89	92		73	59	47	43	32	26	90	82	77	72	67	61
	80	94	91	91	88	86	86	97	82	70	62	51	39	108	100	95	91	87	83
2-Butanone	20	123	133	158	210	217	228		87	80	80	72	71	89	79	76	69	55	56
	40	121	139	155	152	226		82	72	60	60	56	52	102	96	89	83	77	65
	80	105	122	131	123	140	163	103	83	81	74	67	62	109	101	86	93	86	84
2-pentanone	20	86	74	65	60	55	47		64	42	34	24	20	89	78	65	61	55	47

	40	89	86	79	75	70		71	53	40	33	23	19	91	82	72	70	65	59
	80	94	92	81	82	76	70	96	78	59	54	43	36	108	100	91	85	87	82
4-heptanone	20	86	65	51	42	29	26		42	27	22	10	12	82	64	55	51	38	31
	40	87	78	66	60	51		59	38	26	20	16	13	89	73	69	60	55	49
	80	92	87	85	68	60	53	82	60	44	38	24	21	101	84	87	81	77	71
Propanal	20	132	174	208	233	278	299		84	75	75	71	67	96	86	83	76	70	67
	40	118	154	175	194	239		85	78	76	71	66	66	94	89	83	80	76	71
	80	108	121	134	142	156	166	109	98	90	91	85	75	99	93	87	85	81	81
Hexanal	20	104	86	121	139	125	145		72	130	115	98	92	101	100	95	94	91	85
	40	103	101	135	114	129		92	78	78	73	72	72	105	101	98	95	93	91
	80	100	107	123	98	76	126	155	140	115	126	110	125	99	98	99	103	88	95
Octanal	20	101	95	96	80	65	82		72	63	54	45	61	98	66	69	45	50	51
	40	113	85	87	109	80		79	45	63	52	40	51	83	87	85	61	55	50
	80	132	99	95	90	85	89	110	93	86	80	53	53	101	88	77	64	64	52
Furan	20	93	97	97	100	106	107		85	70	60	46	37	86	86	79	75	70	64
	40	90	97	99	102	111		86	81	74	63	54	47	93	87	84	79	76	71
	80	98	94	92	92	92	92	107	101	93	87	78	67	107	102	97	94	93	89
Furan, 2-methyl-	20	88	76	70	65	58	53		77	59	49	34	27	93	82	76	71	66	62
	40	92	88	83	80	78		87	74	65	56	42	34	104	96	90	83	81	76
	80	95	89	84	79	76	73	106	89	87	76	65	56	107	97	86	93	90	87
Furan, 2,5-dimethyl-	20	89	74	57	44	29	22		66	42	26	12	7	91	80	67	62	54	47
	40	86	81	68	58	46		86	68	47	29	13	6	96	86	75	72	66	59
	80	99	91	82	77	67	59	106	93	72	59	37	22	110	100	90	84	87	79
Methyl acetate	20	89	86	79	68	73	71		65	53	47	42	44	81	79	68	64	57	53
	40	94	88	83	79	81		71	61	50	41	29	22	92	85	79	74	67	63
	80	93	88	86	84	80	72	99	85	72	63	47	38	109	102	96	93	89	85
Ethyl Acetate	20	91	80	67	66	54	48		70	54	47	34	29	89	80	73	67	58	53
	40	89	76	74	71	69		79	57	54	46	37	32	100	94	87	79	74	67

	80	93	84	87	78	77	71	100	87	76	67	58	52	103	98	81	89	85	82
n-Butyl acetate	20	84	82	66	64	52	59		71	59	51	45	38	86	78	69	57	50	50
	40	92	86	81	75	64		82	69	60	52	43	39	92	85	76	71	62	57
	80	93	89	82	78	72	69	97	83	69	64	53	41	101	93	86	82	79	73
Dimethyl sulfide	20	87	73	59	50	36	30		69	45	30	16	9	90	86	80	73	67	61
	40	88	76	63	55	46		80	75	63	50	39	31	89	86	80	77	71	69
	80	93	83	78	73	65	61	104	95	85	76	63	55	109	100	96	95	93	89
Methyl propyl sulfide	20	89	76	64	56	44	37		73	47	31	14	7	93	83	79	73	65	59
	40	86	84	75	66	58		85	74	59	48	35	25	91	88	82	76	73	69
	80	95	89	77	77	68	66	104	96	79	73	60	52	107	102	96	94	91	88
Thiophene	20	77	70	58	54	45	40		75	61	52	39	31	86	76	65	59	50	47
	40	82	79	73	65	60		83	72	63	55	46	40	87	80	74	68	62	51
	80	86	84	78	74	68	62	101	93	84	77	68	62	104	100	82	84	79	80
Thiophene, 3-methyl-	20	73	56	43	38	29	24		64	45	37	27	20	83	66	55	48	39	34
	40	83	71	60	53	42		77	61	50	42	33	27	86	75	65	59	52	46
	80	89	78	71	64	57	51	96	83	71	63	52	46	100	93	85	80	74	69
3-Carene	20	85	71	61	54	42	37		88	84	58	48	42	83	84	80	71	57	60
	40	97	89	88	76	67		92	80	79	64	46	36	94	99	95	85	77	62
	80	104	98	93	80	68	66	91	92	91	84	76	69	105	93	99	94	85	75
α -pinene	20	93	82	78	68	58	60		101	95	75	65	57	99	87	84	85	81	88
	40	86	89	77	76	70		99	90	86	80	67	56	93	100	92	87	84	78
	80	99	81	92	82	86	81	115	103	107	100	83	75	105	104	94	99	93	92
p-Cymene	20	77	59	45	38	29	27		67	50	40	34	26	83	75	63	56	44	43
	40	85	77	67	58	50		79	65	54	48	39	30	93	87	75	67	63	51
	80	98	87	78	68	59	54	89	76	69	63	52	46	101	96	88	84	81	65
D-Limonene	20	82	67	57	51	39	41		79	60	50	36	30	81	82	75	70	54	53
	40	87	83	75	71	58		86	77	63	54	39	28	96	92	75	80	71	59
	80	107	98	90	82	68	66	99	79	82	76	54	41	103	100	95	92	86	69

Eucaliptol	20	81	86	70	33	29	36		80	69	63	57	58	96	125	138	142	123	122
	40	74	82	71	65	63		91	86	80	83	75	87	106	108	113	111	109	88
	80	112	107	95	101	90	84	105	96	94	102	87	82	101	108	114	112	114	94
Ethyl ether	20	94	89	82	70	73	69		86	82	79	72	67	88	90	88	85	82	78
	40	96	86	83	80	78		94	90	84	83	76	73	96	92	89	85	87	81
	80	88	86	88	84	80	79	106	103	98	93	91	87	108	103	103	100	99	91
Acetonitrile	20	47	31	27	34	22	17		57	34	31	12	7	57	38	27	24	16	11
	40	69	42	27	32	24		45	26	18	12	8	2	64	47	38	31	23	17
	80	74	50	43	36	27	28	66	39	22	12	4	6	89	69	59	51	40	35
Dimethyl selenide	20	79	65	46	44	30	26		37	12	4	2	1	89	77	65	51	40	31
	40	82	64	54	45	37		78	53	29	17	7	3	96	86	76	64	61	48
	80	87	81	73	71	64	59	96	74	55	40	23	15	106	98	92	86	81	72
Pyrimidine	20	45	26	18	18	12	10		47	27	25	14	13	53	31	21	16	11	10
	40	52	40	30	24	17		36	19	13	11	6	5	65	42	29	25	17	14
	80	80	58	42	38	30	25	56	33	17	17	10	8	86	70	52	44	34	29

Table 6. Comparison of recoveries of volatiles under study for dry and humid test mixtures in Tedlar, Kynar and Flexfilm bags.

VOC		Recovery from Flexfilm bag					Recovery from Kynar bag					Recovery from Tedlar bag				
		[%]					[%]					[%]				
		2 h	4 h	6 h	24 h	48 h	2 h	4 h	6 h	24 h	48 h	2 h	4 h	6 h	24 h	48 h
n-Butane	humid	105	106	108	115	123	105	99	99	95	91	105	106	68	99	95
	dry	115	130	131	202	214	107	101	110	103	85	100	103	103	101	88
n-Pentane	humid	103	104	105	109	114	105	104	103	89	85	103	104	73	99	100
	dry	103	105	109	128	153	101	100	100	78	77	96	98	99	96	95
n-Hexane	humid	103	104	104	111	117	99	101	88	79	76	103	102	81	98	100
	dry	126	151	188	412	583	103	103	99	80	81	102	98	99	102	97
n-Octane	humid	90	92	93	92	91	101	101	100	90	86	90	92	93	92	91
	dry	99	101	99	101	94	103	98	98	87	73	95	98	98	99	95
n-Decane	humid	61	61	61	60	57	82	89	82	82	66	71	61	60	60	57
	dry	84	101	92	90	79	103	97	97	68	52	100	116	104	114	104
Isobutane	humid	102	100	89	73	78	101	105	100	99	83	100	98	89	71	76
	dry	100	98	96	87	94	102	100	94	78	84	94	88	89	86	76
Pentane, 2-methyl-	humid	80	79	79	92	84	104	104	102	92	89	80	79	70	92	84
	dry	100	91	101	115	124	101	98	100	82	75	100	98	97	100	95
Octane, 4-methyl-	humid	96	98	99	106	104	98	98	95	87	90	96	98	79	106	104
	dry	93	108	105	158	189	104	98	96	84	81	103	98	100	103	110
2-Pentene, (E)	humid	91	91	90	90	85	104	101	97	84	42	91	91	92	90	89
	dry	98	98	98	93	83	101	99	99	60	44	96	99	98	98	96

2-Pentene, (Z)	humid	90	102	92	92	93	106	102	97	88	49	90	100	98	92	93
	dry	99	100	98	93	100	94	105	103	57	53	87	86	88	84	87
1-Hexene	humid	99	100	100	104	109	104	102	99	90	78	99	100	73	76	79
	dry	102	98	109	145	168	101	98	98	72	65	101	98	99	98	97
Isoprene	humid	104	104	105	102	99	104	102	99	86	64	98	99	105	97	94
	dry	100	99	98	93	96	100	97	96	60	50	97	98	99	99	95
Benzene	humid	87	86	85	83	81	98	96	94	86	76	87	86	88	83	81
	dry	96	88	95	92	85	98	95	95	74	63	90	97	96	95	92
Toluene	humid	90	89	89	87	84	97	96	93	94	87	90	89	88	87	84
	dry	96	93	95	86	85	97	92	92	80	64	93	96	96	93	84
p-Xylene	humid	86	88	89	82	79	94	90	89	85	74	86	88	89	82	79
	dry	97	96	94	86	79	98	93	91	66	54	80	95	95	90	80
Acetone	humid	99	100	100	98	99	97	92	89	80	66	99	100	97	98	99
	dry	99	97	97	91	88	92	86	84	61	57	98	96	95	93	81
2-Butanone	humid	96	98	97	99	102	106	94	100	100	76	96	98	84	95	97
	dry	115	129	131	140	151	96	93	95	66	71	101	105	89	85	97
2-pentanone	humid	91	93	95	85	83	88	83	78	72	43	91	93	95	85	83
	dry	99	91	97	92	84	94	84	84	52	37	100	99	96	96	88
4-heptanone	humid	87	87	89	64	67	87	87	89	64	67	87	87	89	64	67
	dry	90	100	99	93	86	90	100	99	93	86	90	100	99	93	86
Propanal	humid	97	98	98	97	96	104	104	101	97	97	97	98	93	97	96

	dry	101	102	101	95	89	98	96	96	76	76	100	104	97	95	84
Hexanal	humid	101	95	96	96	109	101	89	95	103	92	100	94	97	96	95
	dry	102	104	102	98	85	116	119	113	85	58	89	100	103	98	90
Octanal	humid	69	92	69	93	76	83	53	50	72	49	81	92	59	93	76
	dry	94	102	101	99	71	105	106	130	86	58	102	98	89	81	73
Furan	humid	90	90	90	88	88	103	103	99	90	77	90	90	93	88	88
	dry	99	97	97	94	88	98	96	95	68	62	99	97	95	97	87
Furan, 2-methyl-	humid	90	91	86	85	81	101	95	93	85	58	90	91	90	85	81
	dry	96	97	95	93	89	99	96	93	60	49	98	96	96	97	92
Furan, 2,5-dimethyl-	humid	93	91	90	79	65	98	89	81	68	23	93	91	92	79	65
	dry	99	96	98	90	83	99	92	94	39	18	98	98	97	95	88
Methyl acetate	humid	90	90	90	86	84	96	93	89	82	68	90	90	92	86	84
	dry	97	97	95	90	88	90	88	86	55	54	97	96	96	93	87
Ethyl Acetate	humid	92	92	91	87	86	98	95	93	87	76	92	92	93	87	86
	dry	95	95	95	92	90	96	92	90	64	59	99	97	99	93	89
n-Butyl acetate	humid	87	92	93	73	78	89	85	81	74	55	87	92	91	85	78
	dry	102	103	98	92	94	97	94	91	53	52	98	98	101	95	82
Dimethyl sulfide	humid	92	92	91	86	80	100	101	104	94	84	92	92	96	86	80
	dry	96	94	95	91	91	94	96	94	63	74	98	97	97	97	90
Methyl propyl sulfide	humid	94	95	93	87	81	104	103	98	90	69	94	95	98	87	81
	dry	96	92	97	92	83	98	93	94	85	75	99	96	95	97	92

Thiophene	humid	89	89	88	83	79	100	97	96	86	77	89	89	88	83	79
	dry	95	88	90	85	81	96	93	91	69	63	100	97	96	92	87
Thiophene, 3-methyl-	humid	87	86	85	77	70	94	90	87	79	63	87	86	87	77	70
	dry	94	87	89	78	73	94	88	86	58	49	94	94	94	88	81
3-Carene	humid	82	82	83	76	69	96	92	90	86	58	82	82	80	76	69
	dry	96	96	91	90	83	99	93	93	72	46	101	96	96	104	98
α -pinene	humid	91	93	95	85	89	92	105	99	92	68	91	93	87	85	89
	dry	94	97	83	94	90	110	100	102	90	57	101	108	104	100	97
p-Cymene	humid	68	69	69	64	59	88	85	84	79	68	68	69	65	64	59
	dry	90	94	88	86	73	96	85	86	72	58	109	105	104	112	98
D-Limonene	humid	68	72	75	66	63	88	85	81	76	36	68	72	72	66	63
	dry	95	97	91	88	80	102	90	91	48	32	100	95	96	100	90
Eucaliptol	humid	88	84	84	45	57	88	84	84	45	57	88	84	84	45	57
	dry	93	107	101	105	96	93	107	101	105	96	93	107	101	105	96
Ethyl ether	humid	90	90	92	89	87	106	106	102	94	88	90	90	96	89	87
	dry	98	96	97	91	93	100	97	96	68	72	96	98	98	99	96
Acetonitrile	humid	72	63	64	59	42	73	62	56	54	30	72	63	69	59	42
	dry	84	80	77	62	43	69	56	52	26	15	87	81	78	64	50
Dimethyl selenide	humid	90	91	90	80	70	107	99	89	80	25	90	91	94	80	70
	dry	94	99	96	93	88	102	97	94	37	18	92	98	98	96	84