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Schmidt, K and Podmore, ID

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Solid Phase Microextraction (SPME) Method Development in Analysis of Volatile Organic Compounds (VOCS) as Potential Biomarkers of Cancer

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Abstract

The analysis of volatile organic compounds [VOCs] is an attractive approach to the discovery of potential cancer biomarkers due to its non-invasive nature and potential low costs of sampling and analysis. Solid phase microextraction [SPME] is one of the main extraction techniques used to date for the collection of VOCs from both in vivo and in vitro samples in studies of potential biomarkers of various types of cancer. It offers simplicity of use, compatibility with both gas-chromatography [GC] and liquid-chromatography [LC] separation techniques and relatively lower costs. Development of the SPME method includes several important considerations: selection of the sampling mode, type of fiber and holder, optimisation of incubation, extraction and desorption conditions, and finally the use of an appropriate calibration procedure. This review summarizes and discusses the particular parameters of the SPME method development used by researchers to date for VOCs collection, from various biological matrices, in search of potential biomarkers of cancer.

Keywords: Cancer biomarker; Method development; Solid phase microextraction; Volatile organic compounds

Introduction

To aid the early detection of cancer which may help in fighting the disease, the discovery of biomarkers is required. Analysis of volatile organic compounds (VOCs) as potential biomarkers of cancer appears to be a very promising approach as it is fast, non-invasive and the cost of sample collection and assay is potentially low. It is thought that the presence of a tumour generates new VOCs, normally not produced by the healthy body, and/or alters the levels of VOCs detected in the body during normal physiological processes. Canine scent detection can distinguish between the various biological samples coming from patients with and without cancer, often with high sensitivity and specificity [1]. Different VOC profiles have been associated with various diseases such as cancer, genetic and metabolic disorders, schizophrenia or infectious diseases [2]. Furthermore, different patterns of VOCs detected in the headspace [HS] of numerous cancerous and non-cancerous cells grown in vitro suggest that potential cancer-specific biomarkers exist [3-5]. The candidate volatile biomarkers, as well as the pros and cons of different biological matrices available for researchers in the quest for the VOC biomarkers of cancer, have been reviewed in ref. [5-10].

The concentrations of the majority of the VOCs occurring in biological samples are relatively low: in the ppm-ppt range in human breath, blood and urine [10-14]. What is more, VOCs are extracted from complex mixtures, therefore before the analysis there is a need for a pre-concentration step to enrich the analytes of interest to a detectable level, as required by the analytical technique to be used. However, pre-concentration itself requires multiple steps, so it is a significant source of errors, resulting in a decrease in the reliability and accuracy of the assay [15]. A decrease in the number of steps of the pre-concentration technique results in better reproducibility and the elimination of interfering compounds. The properties of an ideal device for a sample pre-concentration include simplicity of use, high extraction capacity, high selectivity, speed, efficiency, possible automation and miniaturisation, consideration of the safety of both the environment and the user, and finally compatibility with alternative techniques for separation and detection [16,17]. When compared to the traditional extraction techniques of liquid-liquid extraction and solid-phase extraction, microextraction techniques exhibit some of these properties very well.

Solid phase microextraction (SPME), invented by Pawliszyn and Arthur in 1989 [18], in particular has become very popular in the field of VOC detection in various matrices and various industries due to the simplicity of the SPME device, automation of the process, the absence of a need for solvents, and its compatibility with both gas chromatography (GC) and liquid chromatography (LC) [19]. SPME, next to purge and trap [PT] employing sorbent tubes (followed by thermal desorption), is the main extraction technique used to date for the collection of VOCs in both in vivo and in vitro studies of potential biomarkers of cancer. SPME has been used in many studies as a technique of extraction of VOCs from such human specimens as breath, urine and blood but also from the HS of the cancer cells in vitro in lung, breast, colon, gastric, skin and renal cancers [20-28]. A review discussing the use of HS-SMPE with GC separation for the extraction of VOCs from bio-fluids and bio-materials has been published by Mills and Walker [29]. Reviews looking at the recent advances in SPME techniques for bioanalytical studies have been published [30,31]. Finally, the theory of SPME has been described previously in ref. [32-36].

This review firstly describes briefly the principles of SPME as an extraction technique. Next it gives a tutorial on the steps in SPME method development, whilst also discussing the particular parameters used by the researchers in studies of cancer-associated potential biomarkers. Finally, it briefly examines alternative extraction techniques.

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Principles of SPME Sampling

The SPME technique consists of three steps. Firstly, the sample is placed in the sampling vial (in the case of liquid or solid samples some gas volume is left above it) and the vial is tightly closed. Here the sample is incubated for a specific period of time at a certain temperature. During the second step, the analytes are adsorbed and/or absorbed onto the fiber. The vial is equilibrated at a constant temperature for the time of extraction. The third step is a desorption of VOCs from the SPME fiber in a hot injector port of a gas chromatograph (Figure 1). In the case of high performance liquid chromatography [HPLC] the SPME fiber is introduced into the desorption chamber of HPLC-SPME interface. The interface is a six port injector valve which has a desorption chamber instead of a sample loop. The VOCs are desorbed from the fiber into the mobile phase in a result of either dynamic or static desorption. In dynamic desorption the analytes are desorbed in the moving stream of mobile phase. In static desorption the fiber is soaked in mobile phase for a specific period of time before the compounds are injected onto the column [37].

SPME is a non-exhaustive equilibrium extraction technique, as only a small portion of the target compound is removed from the sample. During extraction, sample molecules preferentially partition between the matrix, headspace and the stationary phase (in the case of a liquid or solid sample), or between the sample and the stationary phase (gas samples) as a result of absorption and/or adsorption process (this depends on the coating type) [34] (Figure 2). With long enough extraction times, an equilibrium concentration of the analyte is established between the two or three phases. When equilibrium is reached, the exposure of the fiber for a longer period of time does not collect any more analyte [32]. The period after which equilibrium is reached depends on the type of the analytes and extraction conditions, and takes from a few minutes to few hours [29]. The partitioning between the three phases (or two in the case of gas samples) depends on the affinity of the analyte to each of them at equilibrium. After the defined period of extraction the fiber is moved to a GC injector or a HPLC chamber interface. SPME also may be used for pre-equilibrium analyte collection.

Because there are two types of SPME coatings [adsorptive (solid) and absorptive (liquid)], the behaviour of the compounds analysed differs (Figure 3). In the process of adsorption, the extraction of the compounds occurs only on the surface of the coating. The analytes are physically trapped or retained by the stationary phase via chemical reactions. The total surface area which is available for adsorption is proportional to the volume of the coating if a constant porosity of the stationary phase is assumed. The amount of analyte adsorbed depends on the initial concentration of the analyte as well as on the concentration of the competitive analyte (competitive displacement reactions). During the absorption process the analytes partition preferentially at equilibrium in the porous material of the liquid polymeric phase. The absorption process also depends on the initial concentration of the analyte in the sample. Again, displacement processes may take place during the absorption, but some studies in water analysis indicate that this is a minor concern in these types of SPME coating [38].

SPME Method Development

Development of the SPME method includes several important considerations: selection of the sampling mode, type of fiber and holder, optimisation of incubation (equilibration), extraction and desorption conditions, and finally the employment of an appropriate calibration procedure [34]. Any given parameter of extraction and desorption must be established experimentally for a given sample and application. These experiments involve the analysis of a series of identical mixtures (samples or prepared mixtures with known amounts of added analytes). The conditions of the extraction and desorption are identical except for the one parameter that is varied. At the end of the series of analyses a plot of the analyte response versus a tested parameter is built and the point where there is no longer an improvement, or there is a lower response, is established. The analysis of the VOCs as potential biomarkers of cancer is usually a profiling of the whole sample and all the analytes present in the sample are of potential interest. Therefore, researchers also compare a tested parameter with the number of analytes detected. A compromise between the various responses for different analytes and the number of analytes detected is reached and this value is used for further studies.

An interesting experimental approach towards SPME method development was applied by Monteiro et al. [22]. They used a central composite design (CCD), a multivariate statistical model, to optimise the extraction conditions of VOCs from the urine of the patients with renal cancer. Instead of performing the evaluation of each of the extraction variables (time of incubation, extraction temperature and
time, salt addition) independently, the combinations among them were defined and employed. CCD enabled for the evaluation of the significance of each of the factors as well as the relationships between them. The number of experiments, time and cost was reduced.

**SPME Device**

The SPME device is composed of the holder and in it, the fiber unit. There are two variants of the SPME holder: one for manual use and one for use with autosamplers or with an HPLC-SPME interface. The fiber assembly consists of a fiber core which is attached via a hub to a stainless steel guiding rod. The guiding rod is enclosed in a hollowed needle that pierces the septum of the vial. During extraction, the fiber is pushed out from this needle and when not in use retracted back. The fiber core is coated with stationary phase (1 or 2 cm long) and made of fused silica (which is relatively easy to break), StableFlex (consisting of 80 µm fused silica with 20 µm plastic polymer, which reduces the chances of breaking the needle but has a thermal limitation of 320 °C), or metal alloy (no thermal limitations; only to use with MicrosealTM septumless systems) [34]. The fiber assemblies for manual use have 24-gauge needles with an outer diameter of 565 µm, which is the smallest possible size that will still retract the 100 µm thick stationary phase. The smallest possible diameters are required for minimal septa coring to prevent losing the sample. However, the 24-gauge needles are very easy to snap while using an autosampler. For these purposes there are more durable 23-gauge needles available with an outside diameter of 646 µm [34]. On the other hand, 23-gauge needles are recommended for use only with septumless systems. Fiber assemblies are used with sample vials with caps containing thin silicone septa, which seal tightly around the needle during piercing.

The profiling of VOCs in the investigation of cancer biomarkers is dominated by the use of manual SPME. In breath analysis, the reasons for the use of manual SPME are the sample size (e.g. 5L or 1L Tedlar bag) and the need for samples of room air to be taken as a control reference. However, there does not seem to be any obvious explanation for its use while working on other matrices, other than the higher cost of an autosampler. Seven studies to date employing an autosampler were conducted on breath and urine matrices (Table 1). The use of an autosampler enables the testing of a larger number of samples (ninety eight 2 mL vials per tray or thirty two 10/20 mL vials per tray), controlled heating and agitation of a sample during incubation and extraction, pre-conditioning of the SPME fiber before each run after desorption, control of the sampling and injection depth of the fiber and finally the opportunity to build the method by employing sequential methods. All of them aid better accuracy and precision [34].

**SPME Sampling Mode**

There are three SPME extraction modes: DI (direct immersion) and HS, in which the fiber is introduced directly into the sample and into the air above the sample, respectively; and a membrane protected mode for dirty samples. The analysis of VOCs is performed either by DI for the gaseous matrix, or HS, which is the most efficient mode for the extraction of analytes from complex liquids and solid samples (with high molecular weight interferences such as proteins) and for the collection of volatile compounds in general [34].

**SPME Fiber Type**

There are four polymers commercially used as SPME stationary phases: divinylbenzene (DVB), polydimethylsiloxane (PDMS), polyacrylate (PA) and carbowax-polyethylene glycol (PEG). They are used on their own (and are available in different thicknesses of a coating) or in combination mixed with carboxen (CAR). The stationary phases differ by polarity (polar, bipolar, non-polar) and extraction mechanism (absorbent or adsorbent). The polarity of the compounds of interest and their molecular weight are factors for choosing of the fiber coating (Table 2).

**Absorbent-type Coatings**

The absorbent is a polymer with liquid properties bonded in various thicknesses to the fiber core. In this type of coating, analytes travel in and out of the stationary phase, which they are attracted to on the basis of their polarity (Figure 3). Retention depends mainly on the thickness of the stationary phase. Larger analyte molecules are retained longer by the coating as they travel through it less quickly than smaller molecules. Coating with thicker phase causes a longer retention of smaller molecules, therefore the choice of the thickness depends on the size of the molecules analysed [34]. There are three commercially available absorbent-type stationary phases: non-polar PDMS, moderately polar PA and polar PEG.
<table>
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<tr>
<th>Reference</th>
<th>Analyzed matrix</th>
<th>Fiber, type of holder, fibers tested</th>
<th>Extraction procedure details</th>
<th>Analytical technique</th>
<th>LOD (and scan mode)</th>
<th>RSD [%]</th>
<th>R²</th>
<th>Scan range [m/z]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abaffy et al. [39] [40]</td>
<td>tissue</td>
<td>F: PDMS/DVB H: manual R: unknown complex matrix: non-polar fiber with broad selectivity T: 75 μm PDMS/CAR</td>
<td>S: 3 mm punch skin biopsy in 1.5 ml vial with 0.3 ml inner tube E: RT (60 min) D: ns D°: 220 °C (1 min)</td>
<td>GC-MS/MS EI DB-SMS column (25 m x 0.2 mm x 0.33 μm)</td>
<td>ns</td>
<td>ns¹ 8.6²</td>
<td>ns</td>
<td>30-300</td>
</tr>
<tr>
<td>Barash et al. [41] [42]</td>
<td>cell culture medium</td>
<td>F: DVB/CAR/PDMS H: manual</td>
<td>S: ultra II SKC badge (with the collected HS emitted from the cells in vitro) placed in a thermal desorption device (volume 350 ml or 750 ml) E: 270 °C (time ns¹, 30 min²) D: 270 °C (time ns)</td>
<td>GC-MS/MS EI HS-SMS column (30 m x 0.25 mm x 0.25 μm)</td>
<td>ns</td>
<td>ns ns</td>
<td>ns</td>
<td>15-220</td>
</tr>
<tr>
<td>Buszewski et al. [43]</td>
<td>tissue</td>
<td>F: 75 μm CAR/PDMS H: manual</td>
<td>S: approx. 2 g of tissue in 20 ml vial I: 40 °C (10 min) E: 40 °C (15 min) D: 200 °C (1 min)</td>
<td>GC-MS/MS EI CPQ column (25 m x 0.25 mm x 3 μm)</td>
<td>0.6-2.8 ppb FS</td>
<td>6-10</td>
<td>0.996-0.999</td>
<td>15-220</td>
</tr>
<tr>
<td>Buszewski et al. [44]</td>
<td>breath, tissue</td>
<td>F: 75 μm CAR/PDMS H: manual</td>
<td>S: 500 μl [breath] S: size ns [tissue] E: 25 °C (10 min) [breath] E: 25 °C (30 min) [tissue] D: 200 °C (time ns)</td>
<td>GC-MS/MS EI CPQ column (25 m x 0.25 mm x 3 μm)</td>
<td>ns</td>
<td>ns ns</td>
<td>ns</td>
<td>15-220</td>
</tr>
<tr>
<td>Chen et al. [45]</td>
<td>breath, cell culture medium</td>
<td>F: 100 μm PDMS H: manual R: VOCs in human breath are non-polar</td>
<td>S: 5L (Tedlar bag) [breath] S: 30 mL in 100 mL glass bottle [cell culture medium] E: 40 °C (time ns) [breath] E: 37 °C (40 min, 1100 rpm) [cell culture medium] D: 260 °C (10 min)</td>
<td>GC-FID DB-1 column (30 m x 0.25 mm x 0.25 μm)</td>
<td>ns</td>
<td>ns ns</td>
<td>n/a</td>
<td>50 - 550</td>
</tr>
<tr>
<td>D’Amico et al. [46]</td>
<td>gauze pads that wiped the skin surface</td>
<td>F: DVB/CAR/PDMS H: ns</td>
<td>S: gauze pad in 20 ml glass vial E: RT (15 h) D: 250 °C (3 min)</td>
<td>GC-MS/MS EI EQUITY-5 column (30 m x 0.25 mm x 0.25 μm)</td>
<td>ns</td>
<td>ns ns</td>
<td>ns</td>
<td>4.2(SIM) 3.6(hexanal) 0.99</td>
</tr>
<tr>
<td>Deng et al. [47]</td>
<td>blood</td>
<td>F: 75 μm CAR/PDMS H: manual T: 100 μm PDMS, PDMS/DVB, 65 μm CW/DVB, PA</td>
<td>S: 5 ml in 15 ml glass vial E: 60 °C (15 min, 1100 rpm) D: 250 °C (30 s)</td>
<td>GC-MS/MS EI HP-SMS column (30 m x 0.25 mm x 0.25 μm)</td>
<td>0.026 nM (hexanal) 0.032 nM (heptanal) SIM</td>
<td>4.2 (hexanal) 3.6 (heptanal) 0.99</td>
<td>ns</td>
<td>41-450 SIM</td>
</tr>
<tr>
<td>Deng and Zhang [48] Deng et al. [49]</td>
<td>blood</td>
<td>F: PDMS/DVB H: manual R: the best reproducibility for extraction of PFBA in aqueous solution than other fibers; aldehydes targeted OFD: 1 ml of PFBHA (17 mg/mL), 25 °C (10 min) S: 1 ml in 8 ml vial E: 60 °C (8 min, 1100 rpm) D: 270 °C (2 min)</td>
<td>GC-MS/MS EI HP-SMS column (30 m x 0.25 mm x 0.25 μm)</td>
<td>0.001-0.006 nM¹ SIM 0.0006 nM² (hexanal) 0.005 nM² (heptanal) SIM</td>
<td>&lt; 6¹</td>
<td>&lt; 8.5²</td>
<td>0.99¹ (hexanal) 0.996² (heptanal)</td>
<td>41-450 SIM</td>
</tr>
<tr>
<td>Fuchs et al. 2010 [50]</td>
<td>breath</td>
<td>F: PDMS/DVB H: manual R: aldehydes targeted OFD: 50 mg of dry PFBHA, 40 °C (10 min) S: 10 ml in 20 ml vial E: 60 °C (8 min) D: 270 °C (1 min)</td>
<td>GC-MS/MS EI MN-SS column (15 m x 0.25 mm x 0.25 μm)</td>
<td>0.0013-0.036 nmoL (29 ppt – 1.3 ppb) SIM</td>
<td>ns</td>
<td>ns ns</td>
<td>SIM</td>
<td>35-280</td>
</tr>
<tr>
<td>García et al. [51]</td>
<td>breath</td>
<td>F: PDMS/DVB H: manual, automatic T: 75 μm PDMS/CAR, PDMS (thickness ns), DVB/CAR/PDMS</td>
<td>S: 5 L (Tedlar bag) E: 25 °C (60 min) D: 175 °C (5 min)</td>
<td>GC-MS/MS EI VF-SMS column (60 m x 0.25 mm x 0.25 μm)</td>
<td>40 ng mL⁻¹, (n-hexane) FS</td>
<td>ns</td>
<td>ns</td>
<td>SIM</td>
</tr>
<tr>
<td>Gaspar et al. [52]</td>
<td>breath</td>
<td>F: 100 μm PDMS H: manual R: targeted non-polar hydrocarbons T: DVB/CAR/PDMS</td>
<td>S: 5 L (Tedlar bag) E: 25 °C (30 min) D: 250 °C (5 min)</td>
<td>GC-MS/MS EI BPXS column (30 m x 0.25 mm x 1 μm)</td>
<td>0.04 - 8.0 ppb (FPD[MS]) FS</td>
<td>9-26 (FPD[MS]) &gt; 0.95 (FPD[MS]) 40-450 (MS) 15-220 (FPD[MS])</td>
<td>40-450 SIM</td>
<td></td>
</tr>
<tr>
<td>Guadagni et al. [12]</td>
<td>urine</td>
<td>F: PDMS/DVB H: manual R: aldehydes targeted S: 3 ml in 10 ml vial (1 g of NaCl, 1 ng mL⁻¹ IS solution) E: 60 °C (20 min, ultrasonic bath) D: 200 °C (time ns)</td>
<td>GC-MS/MS EI CP-PoraBOND Q column (25 m x 0.25 mm x 3 μm)</td>
<td>1.1 pg/μL SIM</td>
<td>0.45-4.46</td>
<td>0.99</td>
<td>30-300 SIM</td>
<td></td>
</tr>
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<table>
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<tr>
<th>Hanai et al. [53] [54]</th>
<th>urine, cell culture medium</th>
<th>F: 2 cm DVB/CAR/PDMS H: automatic T: CAR/PDMS (thickness ns), PDMS/DVB, PA</th>
<th>S: 200 μl in 2 ml vial l: 45 °C (10 min) E: 45 °C (50 min) D: 240 °C (10 min)</th>
<th>GC-MS/EI Inert-Cap Pure-WAX T.L. column (60 m x 2.0 mm transfer line x 0.25 μm)</th>
<th>ns$^1$ 0.004-0.058 μM$^2$ SIM</th>
<th>ns</th>
<th>ns &gt; 0.99$^1$</th>
<th>40-500 SIM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kischkel et al. [55]</td>
<td>breath</td>
<td>F: 75 μm CAR/PDMS H: automatic</td>
<td>S: 10 ml in 20 ml glass vial l: 40 °C (3 min, stirring) E: 40 °C (7 min) D: 290 °C (1 min)</td>
<td>GC-MS/EI CP-PoraBond Q column (25 m x 0.32 mm x 5 μm)</td>
<td>0.023-1.305 ppb SIM</td>
<td>ns</td>
<td>ns &gt; 0.91</td>
<td>35-300 SIM</td>
</tr>
<tr>
<td>Kwak et al. [25]</td>
<td>cell culture medium</td>
<td>F: 2 cm DVB/CAR/PDMS</td>
<td>S: 1 ml in 4 ml vial (750 mg of NaCl, pH 2, 3 or 10) E: 37 °C (30 min, stirring) D: 230 °C (time ns)</td>
<td>GC-MS/EI Stabil wax column (30 m x 0.32 mm x 1 μm)</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>41-400</td>
</tr>
<tr>
<td>Ligor et al. [56]</td>
<td>breath, tissue</td>
<td>F: 75 μm CAR/PDMS H: manual</td>
<td>S: 2.5 g in 20 ml glass vial l: 30 °C (10 min) E: 30 °C (15 min) D: 200 °C (1 min)</td>
<td>GC-MS/EI CP-PoraBond Q column (25 m x 0.25 mm x 3 μm)</td>
<td>1.4-5.0 ppb FS 0.6-0.9 ppb SIM</td>
<td>7-10</td>
<td>0.994-0.999</td>
<td>15-220 SIM</td>
</tr>
<tr>
<td>Ligor et al. [26]</td>
<td>breath</td>
<td>F: 75 μm CAR/PDMS H: manual</td>
<td>S: 18 ml in 20 ml vial l: 37 °C (10 min) E: 37 °C (15 min) D: 290 °C (1 min)</td>
<td>GC-MS/EI Stabil wax column (30 m x 0.32 mm x 1 μm)</td>
<td>0.05 - 15.00 ppb$^1$ FS 0.7 - 17.2 ppb$^2$ FS</td>
<td>na</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>Matsunura et al. [58]</td>
<td>urine</td>
<td>F: 2 cm DVB/CAR/PDMS H: manual</td>
<td>S: 100 μl in 4 ml glass vial l: 40 °C (30 min) E: 40 °C (5 min) D: 230 °C (5 min)</td>
<td>GC-MS/EI Stabil wax column (30 m x 0.32 mm x 1 μm)</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>41-400</td>
</tr>
<tr>
<td>Monteiro et al. [22]</td>
<td>urine</td>
<td>F: PDMS/DVB H: automatic T: DVB/CAR/PDMS, 100 μm PDMS, 7 μm PDMS, PA</td>
<td>S: 2 ml in 10 ml vial (0.59 g of NaCl, pH 2) l: 68 °C (9 min) E: 68 °C (24 min, 250 rpm) D: 250 °C (4 min)</td>
<td>GC-MS/EI VF-5 MS column (30 m x 0.25 mm x 0.25 μm)</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>40-400</td>
</tr>
<tr>
<td>Poli et al. [59]</td>
<td>breath</td>
<td>F: 75 μm CAR/PDMS H: manual</td>
<td>S: 150 ml (using Bio-VOC® breath sampler) E: 22 °C (30 min) D: 280 °C (5 min)</td>
<td>GC-MS/EI Equility™-1 column (30 m x 0.25 mm x 1 μm)</td>
<td>10$^{-12}$M FS 3.1-13.7 &gt; 0.98</td>
<td>40-350</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poli et al. [60]</td>
<td>breath</td>
<td>F: PDMS/DVB H: manual</td>
<td>OFD: 1 ml of PFBHA (17 mg/ ml), RT (10 min, stirring) S: 150 ml (using Bio-VOC® breath sampler) E: RT (45 min) D: 280 °C (time ns)</td>
<td>GC-MS/EI HP-SMS column (30 m x 0.25 mm x 0.50 μm)</td>
<td>0 x 10$^{-12}$ M SIM 7.2-15.1 0.97-0.99 SIM</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Pyo et al. [61]</td>
<td>cell culture medium</td>
<td>F: PDMS/DVB H: manual</td>
<td>S: cell culture medium (volume ns) in cell culture flask (size ns) l: 40 min (220 °C) E: 200 °C (200 °C) D: 200 °C (200 °C)</td>
<td>GC-MS/EI HP-S column (30 m x 0.32 mm x 0.25 μm)</td>
<td>0.04-0.4 ppb SIM 1.02-8.23 &gt; 0.99</td>
<td>40-400</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rudnicka et al. [62] [63]</td>
<td>breath</td>
<td>F: 75 μm CAR/PDMS H: manual</td>
<td>S: 10 ml in glass vial S1: 1 L (Tedlar bag) E1: 25 °C (15 min) E2: 25 °C (15 min) D: 200 °C (2 min)</td>
<td>GC-MS/EI CP-PoraBond Q column (25 m x 0.25 mm x 3 μm) 0.31-0.75 ppb$^1$ FS 3.36-9.54$^2$ &gt; 0.98$^1$</td>
<td>3-10$^2$ &gt;0.99$^2$</td>
<td>30-300</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Silva et al. [27] [28]</td>
<td>urine</td>
<td>F: 75 μm CAR/PDMS H: manual</td>
<td>S: 4 ml in 8 ml glass vial (0.8 g of NaCl, pH 1-2) l: 50 °C (60 min) E: 250 °C (6 min) D: 250 °C (6 min)</td>
<td>GC-MS/EI BP-20 column (30 m x 0.25 mm x 0.25 μm)</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>30-300</td>
</tr>
<tr>
<td>Song et al. [64]</td>
<td>breath</td>
<td>F: 75 μm CAR/PDMS H: manual</td>
<td>S: 4 L (Tedlar bag or glass bottle) E: RT (30 min) D: 250 °C (time ns)</td>
<td>GC-MS/EI RxnTM-SMS column (30 m x 0.25 mm x 0.25 μm)</td>
<td>ns &lt; 6 0.996, 0.988</td>
<td>35-350</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ulanowska et al. [65]</td>
<td>breath</td>
<td>F: 75 μm CAR/PDMS H: manual</td>
<td>S: 500 ml (transferred into 1L Tedlar bag) E: 25 °C (10 min) D: 200 °C (time ns)</td>
<td>GC-MS/EI CPQ column (25 μm x 0.25mm x 3 μm)</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>15-220</td>
</tr>
</tbody>
</table>

Biomarkers Discovery & Validation

Three tables are presented, each with different sample types and analytical conditions. The tables summarize various methods used for analyzing volatile organic compounds (VOCs) in breath, urine, and cell culture medium. The tables include details on sample collection, extraction techniques, and analytical methods used for detection and quantification. The references [53-65] cited at the bottom of the page provide further details on these methodologies. The tables also highlight the use of different columns and detectors such as GC-MS/EI, GC-QMS/EI, GC-ITMS/EI, and others, with specifications for column lengths, diameters, and temperatures. The results section (not shown) would typically present the analytical data and results from these methodologies, including concentrations of various VOCs and their significance as potential biomarkers.
Adsorbent-type Coatings

In adsorbent-type coatings, solid porous polymer, porous carbon or silica is bonded to a liquid material coated on a fiber core. Sample molecules travel into pores of the adsorbent and may interact with its particles via hydrogen bonding. Retention here is based on the size of the sample molecules and then the diameter of the pores and the amount of porosity. Pores are categorised into three types: macro (openings with > 500 Å), meso (openings 20-500 Å) and micro (2-20 Å). A pore retains a sample molecule which is half the size of the pore diameter [34]. There are three commercially available adsorbent-type stationary phases, which were used for the extraction of VOCs: CAR-PDMS, PDMS-DVB and DVB-CAR-PDMS (Table 1).

CAR-PDMS fibers were developed for the extraction of volatile and small analytical molecules. This is because CAR can be produced as a sieve with pores of variable sizes, with the micropores able to retain C3 compounds and semi-volatile analytes. DVB-CAR-PDMS fiber coating is most efficient for the extraction of higher molecular weight compounds and PDMS-DVB is not very efficient for the desorption of lower molecular weight analytes. The ratio of the two solid materials (CAR and DVB) in the DVB-CAR-PDMS fiber was determined by evaluating what thickness of each coating optimised the extraction of different sized hydrocarbons. The amounts of each compound detected with this fiber was between the amounts determined with the CAR-PDMS and the PDMS-DVB fibers, so it can be used for the analysis of a wide range of molecular weight compounds [34].

The analysis of volatiles as biomarkers of cancer was performed in most cases with the use of the 75 µm CAR-PDMS fiber regardless of the type of sample tested (Table 1). This fiber was initially developed for the extraction of volatile and small compounds so its use is justified [34]. Tests for the selection of the fiber with the best efficiency for collecting volatile analytes were conducted in some of the studies in Table 1. Such tests involved the introduction of the fiber into a mixture of known concentrations of VOCs commonly detected in human breath and representing different chemical groups [65]. In these tests 75 µm CAR-PDMS was found most often to be the most efficient in terms of total peak area, number of detected compounds and reproducibility (Table 1). PDMS and PA were the fibers with the least efficiency in these tests. The PA coating has a polar affinity and most VOCs in human breath are non-polar. The PDMS fiber is efficient for the collection of hydrocarbons, but perhaps not other VOCs. Interestingly, only Ulanowska et al. [65] compared two thicknesses of the CAR-PDMS type of coating (the 75 µm being slightly more efficient than 85 µm), which perhaps should be considered as a standard test that should be employed, as CAR-PDMS appears to be the most suitable fiber type for VOCs analysis.

DVB-CAR-PDMS was the second most efficient fiber for VOC extraction from different types of matrix [22,27,28,51,65]. This fiber showed a very wide spectrum of detected compounds in the study of Barash et al. [41], where HS-SPME-GC-MS analysis identified 350-400

Table 1: Demonstrates the analysed matrix, the type of fiber and holder used, the extraction conditions, the applied separation and the detection system, and the achieved parameters in the studies investigating potential biomarkers of cancer performed to date.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Sample</th>
<th>Fiber</th>
<th>Holder</th>
<th>Extraction Medium</th>
<th>Extraction Conditions</th>
<th>Analysis System</th>
<th>Result Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wang et al. [23]</td>
<td>breath, blood</td>
<td>F: 75 µm CAR/PDMS</td>
<td>H: manual</td>
<td>S1: 10 ml [breath] S2: 2 ml in 20 ml vial [blood] D: 200 °C (2 min)</td>
<td>GC-MS/EI DB-SMS column (30 m x 0.25 mm x 0.25 µm)</td>
<td>ns</td>
<td>35-200</td>
</tr>
<tr>
<td>Xue et al. [67]</td>
<td>blood</td>
<td>F: 75 µm CAR/PDMS</td>
<td>H: manual</td>
<td>S: 5 ml in 15 ml vial</td>
<td>E: 60 °C (40 min, 1100 rpm) D: 250 °C (30 s)</td>
<td>GC-MS/EI HP-SMS column (30 m x 0.25 mm x 0.25 µm)</td>
<td>ns</td>
</tr>
<tr>
<td>Yu et al. [68]</td>
<td>breath</td>
<td>F: 75 µm PDMS</td>
<td>H: manual</td>
<td>R: non-polar hydrocarbons targeted, thick phase more suitable for VOCs</td>
<td>GC-FID DB-1 column (30 m x 0.25 mm x 0.25 µm)</td>
<td>1.2 x 10^{-7} – 1.26 ng/ml</td>
<td>&gt; 0.98 n/a</td>
</tr>
<tr>
<td>Yu et al. [20]</td>
<td>breath, cell culture medium</td>
<td>F: ns</td>
<td>H: manual</td>
<td>S: 5 L (Tedlar bag) E: 28 °C (20 min) D: 280 °C (10 min)</td>
<td>GC-MS (mass analyzer ns) column ns</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>Zhang et al. [21]</td>
<td>cell culture medium</td>
<td>F: 75 µm CAR/PDMS</td>
<td>H: manual</td>
<td>S: 10 ml in 20 ml vial</td>
<td>E: 38 °C (10 min) D: 280 °C (2 min)</td>
<td>GC, MS/EI Rx-SMS column (30 m x 0.25 mm x 0.25 µm)</td>
<td>ns</td>
</tr>
<tr>
<td>Zimmermann et al. [24]</td>
<td>cell culture medium</td>
<td>F: CAR/DVB</td>
<td>H: manual</td>
<td>R: expected alcohols, esters and ketones</td>
<td>S: cell culture medium (volume ns) in glass flask (size ns) E: 37 °C (40 min) D: 200 °C (20 s)</td>
<td>GC, MS/EI SB-1 column (60 m x 0.32 mm x 0.2 μm)</td>
<td>ns</td>
</tr>
</tbody>
</table>

CAR: Carboxen; D: Desorption; DVB: Divinylbenzene; E: Extraction; EI: Electron Ionization; F: SPME Fiber type; GC: Gas Chromatography; H: Holder type used; I: Incubation; IT: Ion Trap; LOD: Limit of Detection; M: Matrix; MS: Mass - Spectrometry; nd: not applicable; ns: not specified; OFD: on-Fiber Derivatization; PA: Polyacrylate; PDMS: Polydimethylsiloxane; PFBHA: O-(2,3,4,5,6-Pentafluorophenyl) Methylhydroxylamine Hydrochloride; RSD: Relative Standard Deviation; RT: Room Temperature; Q: Quadupole; R: Retention for the SPME Fiber Selection; S: Sample; SIM: Selected Ion Monitoring; T: SPME fibers that were tested; TOF: Time-Of-Flight; 1 = parameter or result used/obtained in the study or with the use of the matrix with the superscript 1; 2 = parameter or result used/obtained in the study or with the use of the matrix with the superscript 2.

different VOCs either produced or consumed by at least one of the investigated seven lung cancer cell lines. However, this group did not try any other fiber coating. Interestingly, in the only test comparing 75 µm CAR/PDMS with 2 cm DVB/CAR/PDMS fiber, the latter had better efficiency [53, 54].

The PDMS-DVB fiber was selected in studies targeting aldehydes with on-fiber derivatization with O-(2,4,6-trifluoromethyl)hydroxylamine hydrochloride (PFBHA) [48-50, 60]. This fiber was proven to be the most efficient in loading PFBHA, when different fibers (CAR-PDMS among them) were tested in terms of selectivity (mass loading of PFBHA and peak tailing), reproducibility, and ability to retain the largest amounts of PFBHA [69]. PDMS-DVB fiber has also shown very good sensitivity in a study targeting aldehydes without derivatization [12, 22]. The fiber also showed better efficiency than CAR-PDMS for the collection of VOCs in tests where skin tissue was studied [39]. In the study of García et al. [51], both PDMS-DVB and DVB/CAR/PDMS showed a higher number of detected VOCs than 75 µm CAR/PDMS. They had very similar performance with 75 µm CAR/PDMS with 2 cm DVB/CAR/PDMS fiber, the latter had better efficiency [53, 54].

100 µm PDMS was the fiber of choice in studies where non-polar hydrocarbons only were targeted and has shown relatively low limits of detection (LODs) (Table 1). In addition, it was more efficient for these analytes when tested along with 1 cm DVB/CAR-PDMS [52].

**Extraction Time and Temperature**

Extraction time is usually the most time-limiting factor in SPME, and is therefore one of the main parameters to optimise. It might be shortened by efficient agitation of aqueous solutions and/or elevation of temperature. However, although higher temperatures result in the more efficient release of compounds from the matrix, an increase in temperature simultaneously causes loss of sensitivity as distribution constants decrease i.e. equilibrium is reached faster but the amount of analyte extracted is smaller at this equilibrium [34]. Therefore, the selection of the time of extraction is a compromise between the sensitivity, length and repeatability of the method. Agitation reduces the time it takes for equilibrium to be reached, and improves sensitivity in pre-equilibrium extraction, as it enhances the mass transport between the sample and the stationary phase of a fiber.

For the *in vitro* and *in vivo* studies of potential cancer biomarkers various sample times were employed ranging from 15 hr to 10 min (Table 1). They depend on the temperature, sample volume and matrix conditions employed during extraction.

**Matrix Conditions**

Better sensitivity of the SPME method may be achieved by optimisation of the matrix conditions which include sample volume (in the case of HS extraction), temperature, pH, ionic strength, sample agitation and the addition of an organic modifier. Salt addition (increase of ionic strength) and pH adjustment are common techniques for the enhancement of the extraction efficiency of organic analytes from aqueous solutions [34].

**Sample volume**

Phase ratio is the proportion of gaseous volume to the sample volume in the vial. The lower the values of phase ratio, the better the sensitivity of the HS method. Therefore, the HS volume should be as small as possible in order to achieve higher sensitivity, as equilibration time is reduced and the mass of compound extracted by fiber increases thereby improving detection limits [70]. The larger the HS volume, the more of the analysed compound goes into the HS, and the less goes onto the SPME fiber and remains in the liquid phase [71].

**Salt addition**

The ionic strength of the sample is modified by the addition of salt and it may influence the extraction in two ways: modifying the properties of the phase boundary or a “salting-out” effect. The latter refers to the process of decreasing the solubility of hydrophobic analytes in the aqueous phase, and is more often observed. The sensitivity of HS analytical methods is widely enhanced by the “salting-out” effect [71]. The process improves sensitivity through the formation of hydration spheres by water molecules with salt ions. This effect drives the additional sample molecules into the HS due to a reduction in the concentration of water molecules available for dissolving analysed compounds. However with a higher concentration of salts an opposite process may occur. Electrostatic interactions of analyte molecules with the ionic salt molecules in the solution may reduce their movement into the HS [32]. In general, salt addition increases the extraction of polar compounds. However, it has no significant effect on non-polar compounds.

Salt was added to the urine samples in the studies of Silva et al. [27, 28], however they did not test the efficiency of extraction with higher or lower amounts of salt. The optimum amount of salt found with the use of CCD was 0.59 g per 2 ml of urine [22]. The “salting

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**Table 2. Summary of commercially available SPME fibers.**

<table>
<thead>
<tr>
<th>Polymer coating and thickness</th>
<th>Recommended application</th>
<th>Mechanism</th>
<th>MW</th>
<th>Polarity</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 µm PDMS</td>
<td>Volatiles</td>
<td>Absorbent</td>
<td>60-275</td>
<td>Non-polar</td>
</tr>
<tr>
<td>30 µm PDMS</td>
<td>Non-polar semi-volatiles</td>
<td>Absorbent</td>
<td>80-500</td>
<td>Non-polar</td>
</tr>
<tr>
<td>7 µm PDMS</td>
<td>Non-polar high molecular weight compounds</td>
<td>Absorbent</td>
<td>125-600</td>
<td>Non-polar</td>
</tr>
<tr>
<td>60 µm PEG</td>
<td>Alcohols and polar compounds</td>
<td>Absorbent</td>
<td>40-275</td>
<td>Polar</td>
</tr>
<tr>
<td>85 µm PA</td>
<td>Polar semi-volatiles</td>
<td>Absorbent</td>
<td>80-300</td>
<td>Polar</td>
</tr>
<tr>
<td>75 µm/85 µm CAR/PDMS</td>
<td>Gases and low molecular weight compounds</td>
<td>Adsorbent</td>
<td>30-225</td>
<td>Bipolar</td>
</tr>
<tr>
<td>65 µm PDMS/DVB</td>
<td>Volatiles, amines and nitro-aromatic compounds</td>
<td>Adsorbent</td>
<td>50-300</td>
<td>Bipolar</td>
</tr>
<tr>
<td>60 µm PDMS/DVB</td>
<td>Amines, nitroaromatic and polar compounds (HPLC use only)</td>
<td>Adsorbent</td>
<td>50-300</td>
<td>Bipolar</td>
</tr>
<tr>
<td>50/30 µm DVB/CAR/PDMS on a StableFlex fiber</td>
<td>Flavour compounds: volatiles and semi-volatiles, C3-C20</td>
<td>Adsorbent</td>
<td>40-275</td>
<td>Bipolar</td>
</tr>
<tr>
<td>50/30 µm DVB/CAR/PDMS on a 2 cm StableFlex fiber</td>
<td>Trace compound analysis</td>
<td>Adsorbent</td>
<td>40-275</td>
<td>Bipolar</td>
</tr>
</tbody>
</table>

CAR: Carboxen; PDMS: Polydimethylsiloxane; DVB: Divinylbenzene; HPLC: High Performance Liquid Chromatography; PA: Polyacrylate; PEG: Carbowax-Polyethylene Glycol [19].
out" effect was tested in another study, where different amounts of sodium chloride were added to urine samples and then signal to noise ratio values of all the analytes were examined. Salt addition improved the extraction recovery and sensitivity of the method [12]. The blood matrix has not been treated with salts as this could result in clot formation unless the blood sample is deproteinised [34].

pH adjustment

pH adjustment may improve method sensitivity through conversion of the ion species into neutral forms. Only the neutral/undissociated species of compounds are adsorbed/absorbed from the HS by the SPME fiber. Low pH values will benefit the extraction of acidic analytes, and high values will increase the extraction efficiency of basic compounds. Kwak et al. [25] reported that some organic acids were major VOCs detected at low pH value and were barely visible in chromatogram obtained in neutral conditions. On the other hand, the intensities of many other compounds decreased with a reduction of pH. Acidic pH (pH 2) was found to be the optimum, both in terms of the total number of the detected compounds and the total chromatographic peak area obtained, for the extraction of VOCs from the urine samples by Monteiro et al. [22]. Silva et al. [27,28] also adjusted urine samples to 1-2 pH. The group did not test, however, neutral or high pH values to establish the optimum pH of extraction.

Desorption

Desorption times used in VOC studies of cancer vary greatly from 20 s (at 200°C) to 10 min (at 240°C). In general, an increase of the injector temperature reduces desorption time. Maximum fiber coating durability may be achieved by minimizing the temperature and time of desorption. On the other hand both parameters must be compromised to prevent an analyte carry-over. In general, when the temperature of the injector port is 200°C, desorption time is approximately 1 second for a 100 μm coating for low molecular mass compounds such as VOCs. Desorption is successfully carried out by the generation of a high linear flow rate, which ensures that the desorbed compound is removed immediately from the stationary phase of a fiber [34]. As there is no solvent use with SPME, the desorption is usually performed in splitless mode aiding sensitivity [19].

The desorption time has been optimised in one study in Table 2 by determination of the sum of peak areas yielded under different desorption times (10, 20, 30, 40, 60, 90 and 120 s at 250°C) [67]. Holding the SPME fiber in the injector port for longer than 30 s did not result in further improvement of the detector’s response. Therefore 30 s has been chosen for all subsequent experiments.

Calibration

SPME as a non-exhaustive method needs careful selection of calibration for quantitative analysis. Quantification of analytes in SPME is based on the principle that the amount of compound extracted onto the stationary phase is linearly proportional to the compound concentration in the sample. There are several calibration methods available for SPME [72]. Studies of VOCs as cancer biomarkers where quantification has been performed used mainly the external standards method of calibration (Table 1). This is acceptable as long as blank sample matrices are available. These blank samples are breath, blood and urine from healthy patients and pure culture medium for in vitro studies (medium incubated for the same amount of time as the in vitro grown cells). Alternatively a method of internal standard addition may be used. Here, however, one must be sure that a chosen internal standard is affected by matrix in the same way as compounds of interest [73].

In some sample types, such as urine or cell culture medium, matrix effects are expected as the composition of the matrix is not entirely known. Here a standard addition method of calibration should be used. For example Pyo et al. [61] investigated VOCs present in the HS of the lung cancer cells grown in vitro. For method validation the researchers prepared the mixture of standard solution in methanol (external standards method of calibration). Here the slope of the calibration graph could potentially differ from the one obtained with the use of the standard addition method (performed in the cell culture medium as a matrix). This is because the partition coefficients of analytes depend on the composition of the matrix and polarity of the compounds [73]. They are different for methanol and cell culture medium. In addition, methanol could potentially compete with the analytes for the places of absorption and/ or adsorption on the SPME fiber.

Method validation

Once the SPME parameters are optimised, the method should be tested for a particular application. The tests using optimal extraction conditions should include evaluation of the limits of detection (LOD) and quantitation (LOQ), precision and accuracy of the method, method selectivity and linear dynamic range.

There are different definitions of the LOD in literature [12,74,75]. In the studies, where the LOD level was specified, it was calculated on the basis of ion signal to noise ratio = 3 (Table 1). The SPME methods used by researchers in the studies summarised in Table 1 differ in their sensitivity, accuracy and precision. These variations are probably the results of differences in the fiber used, the analytical instrument used for detection and separation of VOCs, and the choice of equilibrium or pre-equilibrium times of extraction.

According to the Food and Drug Administration [75], for the bioanalytical methods the determined precision should not exceed 15% of the coefficient of variation (also known as relative standard deviation, RSD). The coefficient of determination (denoted as R2) indicates how well the data fits a linearity curve. The R2 value for a calibration curve should be ≥0.997 for the linearity of the analytical method to be achieved [76]. The RSD values in most of the studies presented in Table 1 were < 10% indicating a very good level of precision for these SPME methods. SPME experiments that included derivatization were shown to have higher RSD values, probably due to the additional preparation step. The R2 values were > 0.997 for most of the VOCs in these studies showing very good accuracy of the data models.

SPME versus other extraction techniques

The second main technique along with SPME for the collection of VOCs from exhaled breath or the HS of biological specimens in studies of potential cancer biomarkers is PT (called also dynamic headspace). In comparison to PT, SPME involves fewer steps. PT consists of extraction of the analytes onto the sorbent tube, primary desorption, cold focusing, and secondary desorption. SPME is more simple in use, as it involves firstly sorption of the VOCs onto the fiber, and then desorption. SPME’s ease of use makes possible the development of normalised methods and standardisation [77]. Limits of detection obtained in the studies analysing volatiles as potential cancer biomarkers showed similar levels as sorbent tubes (high-medium ppt range in full scan mode) [62,63,78]. However, although new SPME coatings are under development, there are 10 coating types (compatible with GC) commercially available at the moment. In contrast, there are over 20 different sorbent types commercially available for the sorbent tubes. In addition, sorbent tubes may be packed with up to four different sorbents, making it very attractive due to its versatility.
Other drawbacks of SPME include: ease of the breakage of the fiber, stripping of the coatings, and relatively expensive cost of a fiber assembly. Nevertheless, SPME is an attractive alternative to PT due to its lower cost in hardware (no need for a special desorption unit to be installed, nor is there any requirement for the pump or vacuum devices that are required for PT). Sorbent tubes are also relatively expensive. In addition, some sorbents have high affinity to water which may cause column degradation [79].

Other extraction techniques used in the analysis of VOCs in cancer studies include the needle trap device (NTD) and single drop microextraction (SDME) [80,81]. The NTD contains a sorbent trap inside a needle. The analytes present in a liquid or a gas sample can be actively drawn into and out of the needle using a syringe or a pump, or passively be introduced via the diffusion process. This technique (as in the case of PT) is exhaustive and can achieve similar limits of detection to SPME (high-medium ppt range in SIM mode) [56,80]. SDME, in which a small drop of solvent (around 2 µl) is suspended from the tip of the needle where the compounds are extracted from the headspace, offers relatively low costs, simplicity and elimination of carry over. With the use of in-drop derivatization, SDME offers limits of detection in low ppb range (in SIM mode) [82].

Other commercially available microextraction techniques that could be potentially used in the analysis of VOCs in cancer studies include Stir bar sorptive extraction (SBSE) and Monolithic material sorptive extraction (MMSE, also called MonoTraps). Both techniques, as in the case of SPME, rely on partitioning of analytes between a stationary phase and a sample matrix. In SBSE the analytes are extracted by a stir bar, coated with a stationary phase, in the aqueous solution. However, a stir bar has been previously used also for the HS extraction of volatiles [83]. SBSE technology requires relatively expensive instrumentation (a special thermal desorption unit) and the bars are available with three types of coatings: PDMS, PA and ethylene glycol (EG)-silicone. The extraction of VOCs with the use of MMSE can be performed either in a HS or a floating mode. MonoTraps are available in two variants: traps for thermal desorption (which requires a thermal desorption unit) or traps for liquid extraction (which makes this variant of MonoTraps relatively cheap). MMSE is also limited in the types of the sorptive material, as there are two types available: one made of silica and the other made of silica with activated carbon. In environmental analysis the use of SBSE for the collection of VOCs from water has yielded LOD in the low ppb range (in full scan mode) [84]. MMSE has shown similar limits of detection to HS-SPME in the study analysing VOCs in wine (low ppm in full scan mode) [85]. But both SBSE and MMSE could show different LODs when applied for the analysis of VOCs in biological specimens.

Conclusions

The VOCs profile of a biological sample potentially can provide useful information about human health. The composition of the compounds will vary depending on the disease. VOCs may therefore serve as potential biomarkers in cancer detection and screening contributing to its early detection and treatment monitoring of various diseases, cancer among them. SPME is one of the main extraction techniques used in the studies analysing volatiles as potential cancer biomarkers. When the extraction of VOCs as potential biomarkers of cancer is an untargeted analysis and, therefore, all the volatile compounds in the sample are of potential interest, fiber selection tests should be routine for a given type of cancer, cell line, matrix used etc. The optimised parameters of extraction and desorption vary greatly between the studies analysing VOCs-associated with cancer. These parameters depend on the type of the sample, sample size, and analytical technique used. Researchers who performed the tests for the most efficient SPME coating for VOC extraction from different types of matrix in cancer studies, most frequently selected 75 µm CAR-PDMS as the fiber used for further analysis. Use of an autosampler aids reproducibility and quality of analysis. On the other hand, the use of a manual device does not restrict a sample size. SPME is an attractive extraction technique for collection of VOCs from different samples in the studies of cancer as it eliminates the use of solvents, is relatively cheap and simple in use and its sensitivity may be further improved by the development of the new fiber coatings. However, there are competitive extraction techniques available, some of them offering similar limits of detection at a lower price.

References


