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SPME® - VOCS AS POTENTIAL BIOMARKERS OF CANCER

OVERVIEW

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.

Fig. 1. New SPME-FFA fibers, patented and produced by Chromline Srl.

For these reasons, to have a system which allows the replacement in an automatic way of SPME fibers, can be an important tool in the development of a method and in the analysis of samples on which various investigations have to be carried out.

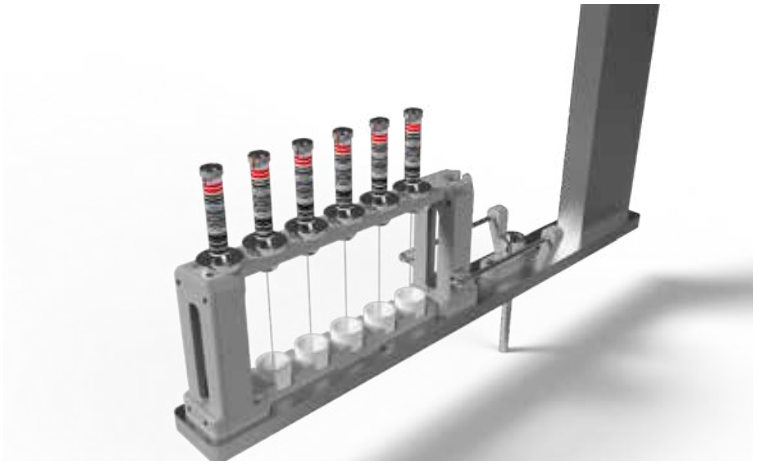

Fig. 2. MFX-6 system.

the basis of ion signal to noise ratio = 3 (Fig. 4). The SPME methods used by researchers in the studies summarised in Figure 4 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, 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. The RSD values in most of the

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

Fig. 3. Summary of commercially available SPME fibers.

Figure 3 show all available fibers and various polymer phases. Each fiber type has an optimum operating range and together they cover a large part of interesting molecules for this type of determination.
MFX System allows all this and with the different configurations as MFX-3, MFX-6 (Fig. 2), MFX-25 and MFX-45 (3, 6, 25 and 45 fibers), has the ability to optimize the work in very different situations.

Now we introduce some application references to show how many different methods of analysis may be of the same sample list using the MFX system, for a complete list of the publications done on biomarkers related to cancer diseases we refer to reviews "*Schmidt K, Podmore's (2015) Solid Phase microextraction (SPME) Method Development in Analysis of Volatile Organic Compounds (VOCs) as Potential Biomarkers of Cancer. J Mol Diagn BioMark 6: 253. doi: 10.4172 / 2155-9929.1000253*".

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.
In the studies, where the LOD level was specified, it was calculated on

studies presented in Figure 4 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.

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

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Reference	Analysed matrix	Fiber, type of holder, fibers tested	Extraction procedure details	Analytical technique	LOD (and scan mode)	RSD [%]	R2	Scan range [m/z]
Hanai et al. [1][1] [2][2]	urine, cell culture medium	F: 2 cm DVB/CAR/PDMS H: automatic T: CAR/PDMS (thickness ns), PDMS/DVB, PA	S: 200 µl in 2 ml vial I: 45 °C (10 min) E: 45 °C (50 min) D: 240 °C (10 min)	GC-$_{TOF}$MS/EI Inert-Cap Pure-WAX T.L. column (60 m + 2 m transfer line x 0.25 mm x 0.5 µm)	ns[1] 0.004-0.058 µM[2] SIM	ns	ns[1] > 0.99[2]	40-500 SIM
Kischkel et al. [3]	breath	F: 75 µm CAR/PDMS H: automatic	S: 10 ml in 20 ml glass vial I: 40 °C (3 min, stirring) E: 40 °C (7 min) D: 290 °C (1 min)	GC-$_{IT}$MS/EI CP PoraBond Q column (25 m x 0.32 mm x 5 µm)	0.023-1.305 nmol/L SIM	ns	> 0.91	35-300 SIM
Kwak et al. [4]	cell culture medium	F: 2 cm DVB/CAR/PDMS	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) S: 10 ml [breath]	GC-$_{Q}$MS/EI Stabilwax column (30 m x 0.32 mm x 1 µm)	ns	ns	ns	41-400
Monteiro et al. [5]	urine	F: PDMS/DVB H: automatic T: DVB/CAR/PDMS, 100 µm PDMS, 7 µm PDMS, PA	NaCl, pH 2) I: 68 °C (9 min) E: 68 °C (24 min, 250 rpm) D: 250 °C (4 min)	GC-$_{IT}$MS/EI VF-5 MS column (30 m x 0.25 mm x 0.25 µm)	ns	ns	ns	40-400
Silva et al. [6] [7]	urine	F: 75 µm CAR/PDMS H: manual T: 100 µm PDMS, PDMS/ DVB, DVB/CAR/PDMS, 70 µm CW/DVB, PA	S: 4 ml in 8 ml glass vial (0.8 g of NaCl, pH 1-2) E: 50 °C (60 min) D: 250 °C (6 min)	GC-$_{Q}$MS/EI BP-20 column (30 m × 0.25 mm × 0.25 µm)	ns	ns	ns	30-300
Wang et al. [66][1] [8][2]	breath, blood[1] blood[2]	F: 75 µm CAR/PDMS H: manual	S[1]: 10 ml [breath] S[1]: 2 ml in 20 ml vial [blood] S[2]: 2 ml, vial size ns E: 40 °C (40 min) D: 200 °C (2 min)	GC-$_{Q}$MS/EI DB-5MS column (30 m x 0.25 mm x 0.25 µm)	ns	ns	ns	35-200
Xue et al. [9]	blood	F: 75 µm CAR/PDMS H: manual T: 100 µm PDMS, PDMS/ DVB, 65 µm CW/DVB, PA	S: 5 ml in 15 ml vial E: 60 °C (40 min, 1100 rpm) D: 250 °C (30 s)	GC-$_{Q}$MS/EI HP-5MS column (30 m x 0.25 mm x 0.25 µm)	ns	5.2	ns	ns
Yu et al. [10]	breath	F: 100 µm PDMS H: manual R: non-polar hydrocarbons targeted, thick phase more suitable for VOCs	S: 5 L (Tedlar bag) E: 26 °C (20 min) D: 280 °C (10 min)	GC-FID DB-1 column (30 m × 0.25 mm × 0.25 µm)	1.2 x 10^{-2} – 1.26 ng/ml n/a	3.7-9.8	> 0.98	n/a
Yu et al. [11]	breath, cell culture medium	F: ns H: manual	S: 5 L (Tedlar bag) [breath] S: cell culture medium (volume ns) in cell culture flask (size ns) E: 37 °C (50 min) [breath] E: RT (100 min) [cell culture medium] D: ns	GC-MS (mass analyzer ns) column ns	ns	ns	ns	ns
Zhang et al. [12]	cell culture medium	F: 75 µm CAR/PDMS H: manual	S: 10 ml in 20 ml vial I: 38 °C (10 min) E: 38 °C (44 min, stirring) D: 280 °C (2 min)	GC-$_{Q}$MS/EI Rx-5MS column (30 m × 0.25 mm × 0.25 µm)	ns	ns	ns	42-400
Zimmermann et al [13]	cell culture medium	F: CAR/DVB H: manual R: expected alcohols, esters and ketones	S: cell culture medium (volume ns) in glass flask (volume ns) E: 37 °C (40 min) D: 200 °C (20 s)	GC-$_{Q}$MS/EI SB-11 column (60 m × 0.32 mm × 0.2 µm)	ns	ns	ns	ns

CAR: Carboxen; D: Desorption; DVB: Divinylbenzene; E: Extraction; EI: Electron Ionization, F: SPME Fiber type Used; FID: Flame Ionization Detector, FS: Full Scan; GC: Gas - Chromatography; H: Holder type used; I: Incubation, IT: Ion Trap, LOD: Limit of Detection; M: Matrix; MS: Mass - Spectrometry; n/a: not applicable; ns: not specified; OFD: on-Fiber Derivatization; PA: Polyacrylate; PDMS: Polydimethylsiloxane; PEG: Carbowax-Polyethylene Glycol; PFBHA: O-(2,3,4,5,6-Pentafluorophenyl) Methylhydroxylamine Hydrochloride; RSD: Relative Standard Deviation; RT: Room Temperature; Q: Quadupole, R2: Coefficient of Determination; R: Reason 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.

Fig. 5. Demonstrates the analysed matrix, the type of fiber and holder used, the extraction conditions, the applied separation and the detection system, and the achieved methodvalidation parameters in the studies investigating potential biomarkers of cancer performed to date.

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

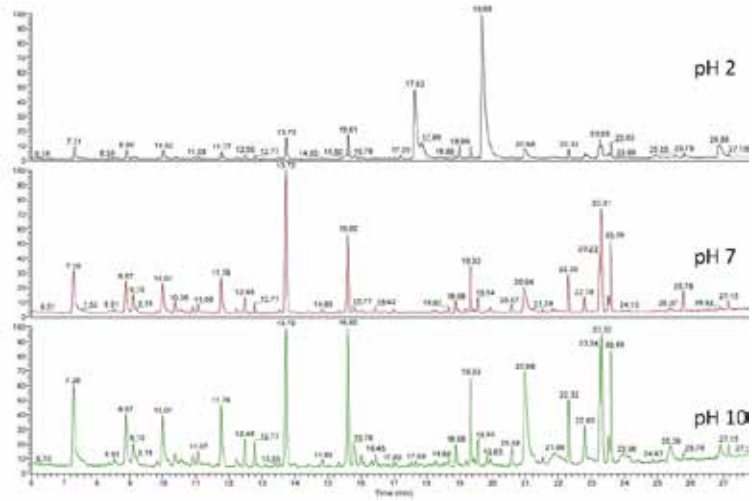

Fig. 4. Effect of pH on the profile of VOCs released from melanoma cell cultures (VPG cells; WM115). Organic acids such as acetic acid (17.63 min) and 3-methylbutyric and 2-methylbutyric acids (19.68 min) that are barely detected at neutral pH become major VOCs when the supernatant is acidified. Reference [4].

extraction from different types of matrix in cancer studies, most frequently selected 75 µm CARPDMS 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.

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