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Ultra-Sensitive Solid-Phase Microextraction–Gas Chromatography–Mass Spectrometry determination of Polycyclic Aromatic Hydrocarbons in snow samples using a Deep Cavity BenzoQxCavitand

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Abstract

A very sensitive and selective solid-phase microextraction-gas chromatography-mass spectrometry method based on the use of a deep cavity BenzoQxCavitand as innovative coating was developed and validated for the simultaneous determination of the 16 US-EPA priority pollutants polycyclic aromatic hydrocarbons (PAHs) in snow samples at ultra-trace levels. The presence of an 8.3 Å deep hydrophobic cavity allowed the engulfment of all the 16 PAHs, providing enhanced selectivity also in presence of interfering aromatic pollutants at high concentration levels. Validation proved the reliability of the method for the determination of the investigated compounds achieving detection limits in the 0.03-0.30 ng/L range, good precision, with relative standard deviations <18% and recovery rates in the 90.8(±2.1)%-109.6(±1.0)%. The detection of low-molecular weight PAHs in snow samples from Antarctica and Alps confirms the widespread occurrence of these compounds, thus assessing the impact of anthropogenic activities onto the environment.

Keywords: solid-phase microextraction, polycyclic aromatic hydrocarbons, priority pollutants, cavitand, trace analysis

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1. Introduction

In spite of the efforts made in the last decades for reducing environmental pollution, the release of compounds related to anthropogenic activities in different compartments is still an ongoing issue, considering the climate warming and ecosystems alteration. Among pollutants, great attention has been paid toward the monitoring of polycyclic aromatic hydrocarbons (PAHs) both to assess the impact of anthropogenic activities onto the environment and to study climate changing (Dat and Chang, 2017; Friedman et al., 2014; Manousi and Zachariadis, 2020; Szopińska et al., 2016; Yu et al., 2019). In fact, PAHs can be generated by natural phenomena like open burning and volcanic activities but their detection in the environment is mostly related to anthropogenic sources, primarily generated by the incomplete combustion of organic matter (industrial, residential and vehicular emissions), or due to petroleum spills (Abdel-Shafy and Mansour, 2016; Alegbeleye et al., 2017; Lawal, 2017; Mojiri et al., 2019). Currently, over 400 PAHs and derivatives have been identified, among which the compounds listed as priority pollutants by the United States Environmental Protection Agency (US-EPA) or the European Environment Agency, which have set strict regulations for the presence of these compounds in the environment (Keith, 2015; Lerda, 2010; Lestingi et al., 2017).

Recent studies demonstrated the occurrence of PAHs in different compartments, such as wastewater, groundswater, surface water, seawater, atmosphere and suspended particulate matter, in populated continents (Mojiri et al., 2019), polar regions-Arctic (Friedman et al., 2014; Yu et al., 2019) and Antarctica (Arcoleo et al., 2021, 2020; Potapowicz et al., 2019; Szopińska et al., 2016; Szumińska et al., 2021; Vecchiato et al., 2015) and high-altitude mountains (Arellano et al., 2018, 2014; Fernandez et al., 2021; Gabrieli et al., 2010; Guzzella et al., 2016), thus highlighting their environmental ubiquity. Being semi-volatile persistant organic pollutants (POPs), PAHs can be easily absorbed onto the aerosol organic matter, leading to long-range atmospheric transport from the emission source to remote areas. Ambient temperature is one of the main parameters determining the deposition of semivolatile compounds: the drop of temperatures lead to the cold condensation of the aerodispersed organic matter and the deposition of the sorbed organic compounds, which are then accumulated in the snow deposits and cryosphere of high-altitude and high-latitude areas (Arellano et al., 2014; Cao et al., 2018; Szopińska et al., 2016; Vecchiato et al., 2018, 2015). Snowpacks can be therefore considered as temporary reservoirs of pollutants: seasonal melting leads to PAHs transfer to both soils and freshwater systems, becoming a serious treat to mountain and polar ecosystems. In addition, depending on temperature, these compounds can be transferred to deeper layers and preserved in glacial ice for long period or released during seasonal thawing. The increase of global temperatures related to climate warming is therefore a long-term hazard concern, being these compounds released from perennal ice and accumulated in biota (Szumińska et al., 2021). Besides the long-rage transport, it was demonstrated that increased concentration levels of pollutants could be also associated with local sources of contamination related to human activities, such as Antarctica research stations (Arcoleo et al., 2021; Potapowicz et al., 2019; Szopińska et al., 2016; Szumińska et al., 2021; Vecchiato et al., 2015).

The analysis of PAHs levels is of pivotal importance in order to assess the impact of the anthropogenic activity on the local ecosystem, to study the long range transport mechanisms and for paleoclimatic studies. The major issues are related to the very low concentration present in these samples (in the ng/L level) and the lack of functional groups in the PAHs structure that can be effectively targeted for their binding. Therefore, proper sample pretreatment is required to preconcentrate the analytes and remove possible interfering compounds prior to the instrumental analysis (Jinadasa et al., 2020; Manousi and Zachariadis, 2020; Ncube et al., 2018; Raza et al., 2018; Sajid et al., 2021). Solid phase extraction and liquid-liquid are the two conventional approaches for the extraction and preconcentration of the analytes from snow and water samples (Jinadasa et al., 2020; Manousi and Zachariadis, 2020; Ncube et al., 2018; Raza et al., 2018; Sajid et al., 2021). However, these techniques present several drawbacks, including time-consuming extraction steps, reduced automation, increased sample handling and large amounts of both sample and organic solvents. Being able to combine a significant reduction of sample volume, automation and minimal sample handling with no or very limited use of organic solvents, micro-extraction techniques have emerged (Manousi and Zachariadis, 2020; Ncube et al., 2018; Raza et al., 2018). Different miniaturized sample preparation techniques have been successfully applied for the extraction and preconcentration of PAHs from environmental matrices, including solid-phase microextraction (SPME), magnetic solid-phase extraction, stir bar sorptive extraction, micro-extraction in packed sorbent (MEPS) and dispersive liquid-liquid micro-extraction (Jinadasa et al., 2020; Manousi and Zachariadis, 2020; Ncube et al., 2018; Raza et al., 2018; Sajid et al., 2021).

Since its development in 1997 (Pawliszyn, 1997), SPME has been considered one of the most intriguing microextraction techniques due to its semplicity, miniaturization, portability, automation, wide range of linear response and solvent-free approach. Currently, several commercial devices based on polymeric coatings are available, covering a wide range of polarity. However, being mostly based on aspecific hydrophobic and π-π interactions, commercial materials have very low selectivity toward a specific class of analytes, thus presenting limited enrichment capabilities. In order to overcome these limitations, novel sorbents have been proposed to be used for environmental monitoring, such as molecular imprinted polymers, ionic liquids, carbon nanotubes, metal organic frameworks and sol-gel based coatings (Delińska et al., 2021; Gómez - Ríos et al.2019; Murtada, 2020; Xu et al., 2013; Zheng et al., 2018).

In this context, the use of molecular receptors as SPME coatings is very attractive to address both selectivity and sensitivity issues: highly selective host molecules could be designed and developed to obtain specific host-guest interactions toward target analytes (Bertani et al., 2016; Bianchi et al., 2014, 2008, 2003; Gómez ‐ Ríos et al., 2019; Li, 1999; Riboni et al., 2016; Trzciński et al., 2017). Our research group has already demonstrated the superior performances of designed quinoxaline cavitands for the extraction of aromatic analytes from environmental samples (Amorini et al., 2022; Bertani et al., 2016; Bianchi et al., 2014, 2008, 2003; Riboni et al., 2016; Trzciński et al., 2017). In the present study, a deep cavity cavitand, the BenzoQxCav is proposed as coating for the solid phase microextraction-gas chromatography-mass spectrometry (SPME-GC-MS) determination of the 16 US-EPA priority PAHs at trace levels in snow samples. After optimization by experimental design, the method was validated and applied for the analysis of snow samples from Antarctica and Italian Alps.

2. Experimental

2.1 Fiber preparation

BenzoQxCav was synthesized and characterized as reported in Amorini et al. (Amorini et al., 2022). Details regarding chemical and materials are reported in **S2.1**.

The BenzoQxCav-based coatings were prepared by applying the dipping procedure already developed in our labs (Bianchi et al., 2014; Riboni et al., 2016): briefly, bare fused silica fibers were dipped into a hydrofluoric acid solution (40% v/v) for 10 s and immediately washed in Milli-Q water. The fiber coating was obtained by vertically dipping the activated silica fiber in the

Duralco 4460 epoxy glue and, after 2 min, in the BenzoQxCav powder (~40 mesh). Details regarding fiber morphology characterization and bleeding tests are reported in **S2.2**.

2.2 GC-MS analysis

Analytes were extracted by direct immersion of the developed SPME fiber in 9.5 ml sample solutions. The analysis was performed by gas chromatography-mass spectrometry (GC-MS), thermally desorbing the fiber directly in the GC-injector port at 300 °C. Internal standard method was used to improve method precision using the deuterated forms of 16-PAHs. Signals were acquired in time scheduled selected-ion monitoring mode (SIM), acquiring 3 ions per analytes, one used for quantitation and two for confirmatory purpose. Details regarding the GC-MS conditions are reported in supplementary data (**S2.3**).

2.4 Method optimization

The extraction conditions of the BenzoQxCav-coated fibers were optimized considering as main factors the extraction time and temperature performing a Box-Wilson central composite face centered design (CCF) to investigate the significant effects and applying the multicriteria method of the Derringer's desirability function approach to find the global optimal experimental conditions. Detailed information are reported in **S2.4**.

2.5 Method validation

Method validation was performed according to EURACHEM guidelines (Magnusson and Örnemark, 2014) under the optimized conditions using not contaminated tap water as blank matrix. Detection and quantitation limits were obtained based on the mean blank response and the standard deviation of fortified blank matrix. Calibration curves were evaluated over three orders of magnitude, in the LOQ-100 ng/L range. Repeatability and batch-to-batch intermediate precision were evaluated as relative standard deviations (RSD%) of the responses of a blank fortified matrix at low, intermediate and high concentration levels. Trueness was calculated for each analyte in terms of recovery rate (RR%) at three concentration levels. Selectivity was evaluated by analyzing water samples containing 70 ng/L of each PAH spiked with a mixture of benzene, toluene, ethylbenzene and p- and o-xylene (BTEX) at the concentration of 1 μg/L each. The responses of PAHs in the BTEX-free and spiked water samples were compared by performing a Student t-test. Finally, the enrichment factors (EFs) of BenzoQxCav-coated fibers were obtained by analyzing a fortified matrix spiked with 25 ng/L of each PAH under the optimized extraction conditions. The results were compared with those of commercially available 30 μm PDMS fibers. Method validation additional information are reported in **S2.5**.

2.6 Analysis of surface snow samples from Antarctica and Italian Alps

The validated SPME-GC-MS method was finally applied for investigating the presence of PAHs in surface snow samples collected by the National Antarctic Research Program (PNRA) in the coastal area of Victoria Land (Antarctica) during the 2020–2021 austral summer. In particular, the analyzed snow samples were collected as represented in **Figure 1**: sample A nearby the meteorological station of *Icaro Camp* (74°42'37" S, 164°07'05" E); sample B in the area of the scientific research base *Enigma Lake* (74°42'38" S, 164°01'10" E); sample C north of Zucchelli station (74°36'55" S, 163°56'29" E); sample D near Cape Phillips (73°03'41" S, 169°36'16" E).

Figure 1. Sampling sites of surface snow in Antarctica.

Alpine snow samples were collected in the Dolomites alpine area in the following locations: sample 1 (46°25'13" N, 11°37'49" E), sample 2 (46°17'55" N, 11°48'16" E), sample 3 (46°19'29" N, 11°44'43" E), sample 4 (46°19'11" N, 11°48'12" E), sample 5 (46°23'41" N, 11°44'26" E) and sample 6 (46°23'0" N, 11°47'0" E) (**Figure 2**). Samples were taken in December 2021. Snow samples were manually sampled after removal of the first 10 centimeters of the snow to prevent contamination using solvent rinsed-glass bottles (100 mL volume). All bottles were rinsed twice with deionized water and dried prior sampling.

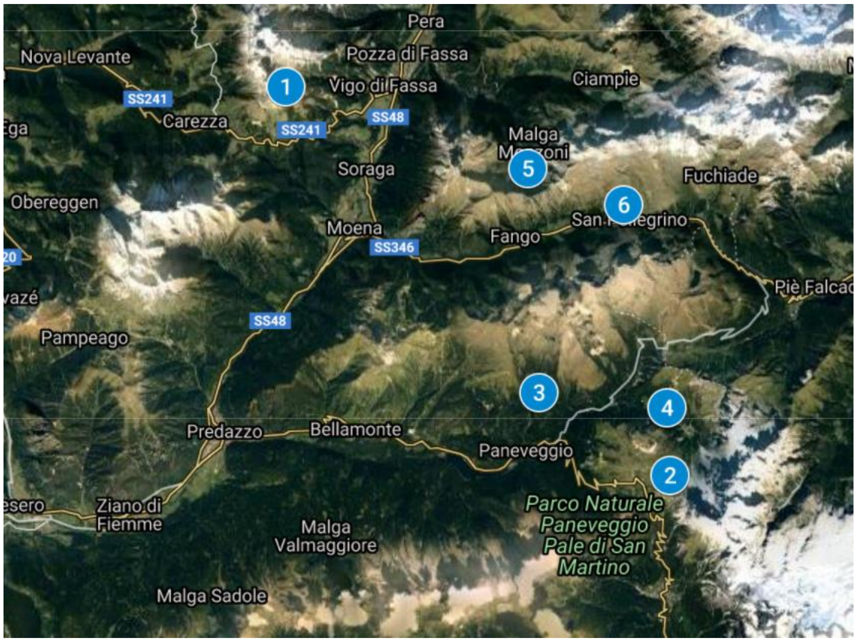

Figure 2. Sampling sites of surface snow in Dolomites.

Samples were delivered and kept at -20 °C until analysis, thawed under chemical hood in an organic-free environment at ambient temperature in dark boxes in order to prevent solar irradiation. The samples were then spiked with the ISs and submitted to analysis. Three replicate measurements per sample were performed.

3. Results and discussion

Starting from the QxCav receptor (Bianchi et al., 2008, 2003), quinoxaline cavitands have demonstrated superior capabilities for the extraction of aromatic guests, being able to bind target molecules by hydrophobic, π-π and CH-π interactions. The enhanced selectivity can be achieved by exploiting specific interactions with functional groups of the target molecule (Bianchi et al., 2014), promoting its confinement inside the cavity (Bertani et al., 2016) and/or by conformationally blocking the cavity to strengthen the interactions between the host and the guest (Riboni et al., 2016; Trzciński et al., 2017).

Since PAHs do not present functional groups that can be effectively targeted for specific binding, BenzoQxCav (**Figure 3)** was synthesized to maximize π-π and CH-π interactions of the analytes with the cavity walls by creating a 2.5 Å deeper cavity compared to the QxCav precursor (Amorini et al., 2022).

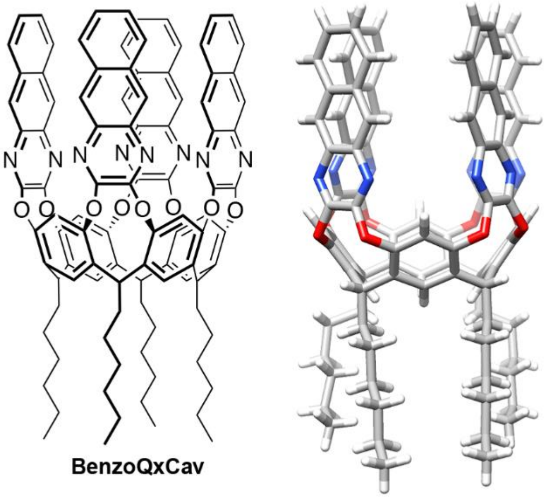

Figure 3. Left: structure of the BenzoQxCav; right: BenzoQxCav molecular structure

In the present study, the inclusion capabilities of the BenzoQxCav were exploited to develop a SPME coating for trace determination of the 16 US-EPA priority pollutants in snow samples. The previously demonstrated high thermal resistance of BenzoQxCav (Amorini et al., 2022) is required to allow the thermal desorption of the analytes in the GC injector port. Coating thickness and morphology were investigated by means of SEM under different magnifications: a homogeneous, porous and uniform coating (**Figure S1**) was observed with an average thickness in the 17-22(±4) μm range (batch of 3 fibers, 3 measured points per fiber). In order to evaluate the background content of PAHs in the coating, the fibers were desorbed at 300 °C, demonstrating the absence of the target analytes in the sorbent material itself.

3.1 SPME Optimization

Based on previous knowledge and the instrumental limitation, the experimental domain was studied as follows: i) extraction time in the 15-45 min range, considering 15 min as the minimum time for an effective adsorption of the analytes and 45 as maximum time to avoid prolonged analysis time; ii) extraction temperature ranging between 40 and 80 °C: 40 °C were selected to obtain a stable thermostating, whereas 80 °C was selected as maximum temperature to avoid both the desorption of the analytes from the coating and the increase of the pressure inside the vials. Salt addition was not optimized since it is known that a reduction of fiber lifetime can occur when immersion analyses are performed, due to the formation of crystals onto the fiber.

For each analyte, the significance of linear, quadratic and interaction effects was evaluated using a forward search step-wise variable algorithm (p to remove 0.05). The obtained models are shown in **Table S1**.

The response surfaces for fluorene, anthracene, fluoranthene, pyrene, benzo[*b*]fluoranthene and dibenzo[*a*,*h*]anthracene are represented in **Figure S2**. As shown, no common trend of the response surfaces is present for the different PAHs, therefore a multicriteria method for identifying the global optimal extraction conditions is required.

By applying the Derringer's desirability function approach, the optimal global extraction conditions resulted in an extraction temperature of 80 °C and an extraction time of 45 min. A global desirability value D=0.81 and single desirability values in the 0.67-0.93 range were calculated, thus providing a good extraction of all PAHs. The achieved findings proved that a global response enhancement was obtained when a long extraction time was applied; as for the temperature, an opposite behavior was observed for the low- and high-molecular weight PAHs, respectively, being the low-molecular weight compounds more easily desorbed by the fiber coating than the heaviest ones. These findings can be rationalized considering that a temperature rise is able to increase the overall extraction kinetic, enhancing the analyte diffusion coefficients (Xu et al., 2020; Zhang et al., 2018) and the BenzoQxCav breathing, thus facilitating the intake of the bulky analytes.

3.5 Method validation

The validation of the developed SPME-GC-MS method allowed to achieve LODs and LOQs values obtaining values in the 0.03-0.30 and 0.09-1.01 ng/L range, respectively. The achieved LODs were better or comparable with the lowest limits reported in previously published studies related to the extraction of PAHs using different extraction techniques and coating materials (Jalili et al., 2020; Jinadasa et al., 2020; Ncube et al., 2018; Raza et al., 2018; Sajid et al., 2021). Additional advantages rely on the reduced sample handling with a limited sample volume, thus minimizing the risk of contamination and reducing the analytical variability.

Method linearity was demonstrated for all PAHs over three order of magnitude (**Table 1)**.

Table 1: LODs, LOQs and regression coefficients for the 16 US-EPA PAHs.

Analyte	**LODs** (ng/L)	**LOQs** (ng/L)	**Regression coefficient*** b(±s_b)
Naphthalene	0.09	0.29	0.1408(±0.0054)
Acenaphthylene	0.21	0.71	0.0984(±0.0042)
Acenaphthene	0.24	0.80	0.0962(±0.0027)
Fluorene	0.07	0.23	0.0670(±0.0017)
Phenanthrene	0.03	0.09	0.0684(±0.0020)
Anthracene	0.07	0.23	0.0662(±0.0026)
Fluoranthene	0.03	0.09	0.0636(±0.0019)
Pyrene	0.03	0.09	0.0716(±0.0021)
Benzo[*a*]anthracene	0.13	0.42	0.0641(±0.0023)
Chrysene	0.09	0.31	0.0622(±0.0015)
Benzo[*b*]fluoranthene	0.10	0.34	0.0664(±0.0018)
Benzo[*k*]fluoranthene	0.11	0.38	0.0872(±0.0035)
Benzo[*a*]pyrene	0.30	1.01	0.0773(±0.0021)
Indeno[1,2,3-*c*,*d*]pyrene	0.14	0.47	0.0697(±0.0023)
Dibenzo[*a*,*h*]anthracene	0.10	0.34	0.0506(±0.0010)
Benzo[*g*,*h*,*i*]perylene	0.17	0.57	0.0831(±0.0022)

*Calibration curve: y=bx

As for method precision, both repeatability and intermediate precision provided excellent results with RSDs always lower than 18% (**Table S2**). ANOVA was applied to evaluate the presence of significant differences among the 3 days of analysis used for evaluating intermediate precision, thus highlighting that mean values were not significantly different (*p* values > 0.05). Excellent results were also found in terms of trueness, obtaining recovery rates in the 90.8(±2.1)-109.6(±1.0)% range, thus assessing the efficiency of the developed SPME-GC-MS method (**Table S2**).

Finally, the selectivity of the devised method was investigated by extracting the target analytes in presence of a mixture of BTEX having a concentration 14 times higher than that of PAHs. The results were then compared with those achieve by extracting the PAHs in a blank fortified matrix without BTEX. As illustrated in **Figure S3**, no significant difference was observed between the GC responses, thus demonstrating the high selectivity of the developed coating. In fact, despite the high concentration of the interfering compounds and their possible interaction adsorption via hydrophobic, π-π and CH-π interaction, the BenzoQxCav coating was characterized by a very high selectivity towards the investigated analytes. This behavior can be rationalized by recalling that the number of π-π and CH-π interactions of the analytes with the cavity walls is responsible of the overall binding. This number is higher for PAHs than for BTEX in the case of the deep cavitand BenzoQxCav, since the interaction with the resorcinarene moiety at the bottom of the cavity is essential to promote the binding (Vincenti and Dalcanale, 1995). Regarding the selectivity of the material toward PAHs in the presence of other hydrophobic compounds of environmental concern, like polychlorinated biphenyl compounds, it is possible to hypothesize an interaction with the supramolecular receptor mainly via π-π interactions; by contrast, as for nitro-PAHs, it can be hypothesised that nitro derivatives are less retained due to the lower affinity for the cavity of the nitro groups and to the additional bulkiness related to the introduction of nitro groups in PAH.

The extraction performance of the BenzoQxCav-coated fibers was investigated in terms of enrichment factors (EFs). The EFs of the developed fibers were in the 10260(±340)-125500(±4300) range and resulted to be generally higher than those obtained by using the PDMS fiber, which varied in the 1420(±42)-43800(±4400) range. As for the extraction mechanism, it has to be considered that an adsorption mechanism is present in the case of the BenzoQxCav due to the presence of a solid porous coating, whereas an absorption mechanism is associated to the use of PDMS fibers, being the analytes mainly extracted by partitioning into a "liquid-like" phase. Finally, the stability of the BenzoQxCav-coated fiber was demonstrated by performing over 150 analyses, without a significant loss in the extraction performance.

3.7 Analysis of Antarctica and Alpine snow samples

Considered the excellent performance in terms of sensitivity, the SPME-GC-MS method based on the BenzoQxCav coated fiber was applied for the analysis of surface snow samples from both Antarctica and Alps. PAHs in the surface snow samples showed total concentrations (Σ16PAHs) in the 32.2-49.5 ng/L range (**Table 2).** Accrding to already published results, PAHs were detected in all samples, thus demonstrating the widespread presence of these compounds in the Antarctica region (Arcoleo et al., 2021, 2020; Potapowicz et al., 2019; Szumińska et al., 2021; Vecchiato et al., 2015). Naphtalene was detected in all the investigated samples with concentration levels ranging from 8.11(±0.21) to 12.36(±0.98) ng/L, accounting for the 20-37% of the total PAHs concentration. Three- and four-ring PAHs consisted of 62-78% of the Σ16PAHs; by contrast, the five- and six-rings PAHs were present in very low amounts in only 2 samples out of 4. Benzo[*a*]anthracene was detected only in samples A and B, respectively, thus accounting for the 8.2 and 16% of the Σ16PAHs present in the two sampled sites.

As previously reported, the presence of high leves of low-molecular weight compounds, namely naphtalene, acenaphthene, fluorene and phenanthrene, could be related to fuel combustion from both local and long range sources (Arcoleo et al., 2021; Szumińska et al., 2021; Vecchiato et al., 2015). Regarding high-molecular weight PAHs, considering that these compounds are less prone to long-range transportation (Szumińska et al., 2021), their presence can be ascribed to local combustion phenomena. Sample A was characterized by the highest levels of pollution among the investigated samples, with ultra-trace amounts of benzo[*a*]pyrene, indeno[1,2,3-*c,d*]pyrene, dibenzo[*a,h*]anthracene and benzo[*g,h,i*]perylene. This sample was collected nearby the meteorological station of *Icaro Camp*, which is close to the Zucchelli research station. Therefore the presence of the heaviest PAHs could be ascribed to local combustion sources (Arcoleo et al., 2021; Szumińska et al., 2021; Vecchiato et al., 2015). Compared to the results published by Arcoleo et al. (Arcoleo et al., 2021), who analyzed samples collected in the same areas, higher levels of Σ16PAHs were obtained. Although the concentrations of the low-weight analytes were very similar, the proposed method being characterized by LOQs lower than those of the MEPS-GC-MS method, allowed a more sensitive detection of five and six-rings PAHs.

Table 2: Concentration (ng/L) of the 16 monitored US-EPA PAHs in the Antarctica snow samples

	Sample A	Sample B	Sample C	Sample D
Naphthalene	12.36(±0.98)	8.56(±0.34)	11.89(±0.30)	8.11(±0.21)
Acenaphthylene	1.05(±0.03)	n.d.	n.d.	3.59(±0.12)
Acenaphthene	1.09(±0.06)	1.38(±0.04)	0.96(±0.06)	1.22(±0.06)
Fluorene	10.18 (±0.13)	7.90(±0.48)	6.72(±0.38)	8.26 (±0.38)
Phenanthrene	2.04(±0.05)	2.19(±0.03)	1.34(±0.04)	1.71(±0.06)
Anthracene	0.66(±0.02)	n.d.	n.d.	n.d.
Fluoranthene	5.61(±0.29)	6.26(±0.32)	4.91(±0.25)	5.35(±0.31)
Pyrene	4.21(±0.29)	5.64(±0.38)	5.89(±0.28)	4.09(±0.19)
Benzo[*a*]anthracene	4.06(±0.13)	6.77(±0.30)	n.q.	n.d.
Chrysene	2.59(±0.06)	3.18(±0.09)	n.d.	n.d.
Benzo[*b*]fluoranthene	n.d.	n.d.	n.d.	n.d.
Benzo[*k*]fluoranthene	n.d.	n.d.	n.d.	n.d.
Benzo[*a*]pyrene	2.21(±0.32)	n.d.	n.d.	n.d.
Indeno[1,2,3-*c*,*d*]pyrene	1.56(±0.06)	n.q.	n.q.	1.13(±0.02)
Dibenzo[*a*,*h*]anthracene	n.d.	n.d.	n.d.	n.d.
Benzo[*g*,*h*,*i*]perylene	1.89(±0.11)	n.q.	n.q.	1.16(±0.03)

n.d.: not detected (<LOD)

n.q.: not quantitated (<LOQ)

The method was then applied for the analysis of surface snow samples collected at high altidute in the Italian Alpine area (over 2000 m, except for Sample 4, which was sampled at 1824 m). All the sampling sites could be reached only by foot and were located far from traffic areas except for Sample 6, which is located nearby an alpine pass. The obtained results are showed in **Table 3**.

Table 3: Concentration (ng/L) of the 16 monitored US-EPA PAHs in the Alpine snow samples

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Naphthalene	4.17(±0.19)	5.62(±0.38)	3.87(±0.19)	12.7(±0.69)	8.86(±0.33)	16.6(±0.71)
Acenaphthylene	n.d.	0.84(±0.03)	n.d.	1.12(±0.04)	n.q.	2.21(±0.05)
Acenaphthene	n.q.	n.d.	n.q.	0.96(±0.05)	n.q.	2.52(±0.11)
Fluorene	1.74(±0.04)	1.12(±0.06)	0.94(±0.02)	1.96(±0.11)	2.07(±0.05)	3.32(±0.11)
Phenanthrene	2.21(±0.09)	2.08(±0.04)	1.73(±0.05)	6.67(±0.23)	4.10(±0.22)	8.83(±0.12)
Anthracene	0.27(±0.02)	n.d.	n.q.	0.35(±0.01)	0.48(±0.02)	0.41(±0.01)
Fluoranthene	2.88(±0.04)	3.02(±0.12)	1.97(±0.15)	9.88(±0.09)	5.26(±0.16)	8.20(±0.23)
Pyrene	1.96(±0.04)	2.54(±0.09)	1.68(±0.03)	6.01(±0.45)	3.81(±0.10)	5.54(±0.13)
Benzo[a]anthracene	n.q.	0.52(±0.03)	n.d.	0.98(±0.03)	0.62(±0.01)	0.72(±0.01)
Chrysene	0.54(±0.04)	0.38(±0.01)	n.d.	0.74(±0.04)	1.76(±0.05)	2.74(±0.10)
Benzo[b]fluoranthene	0.44(±0.03)	n.q.	n.d.	2.69(±0.11)	0.98(±0.06)	2.27(±0.05)
Benzo[k]fluoranthene	0.47(±0.01)	n.d.	n.d.	0.54(±0.01)	n.q.	0.97(±0.01)
Benzo[a]pyrene	n.d.	n.q.	n.d.	n.q.	n.d	1.31(±0.07)
Indeno[1,2,3-c,d]pyrene	n.d.	0.48(±0.02)	0.52(±0.02)	1.24(±0.02)	0.68(±0.05)	1.56(±0.12)
Dibenzo[a,h]anthracene	n.d.	n.d.	n.d.	1.15(±0.04)	n.d.	0.88(±0.08)
Benzo[g,h,i]perylene	n.d.	n.q.	n.d.	0.65(±0.02)	n.d.	1.05(±0.08)

n.d.: not detected (<LOD)

n.q.: not quantitated (<LOQ)

The obtained results are in agreement with the findings reported in literature by Arellano et al. (Arellano et al., 2018, 2014) and Gabrieli et al. (Gabrieli et al., 2010) regarding the concentrations of PAHs in snow samples collected in the alpine areas. As previously reported, the compound with the highest concentration is naphthalene, which was detected in all samples in the 3.87(±0.19)-16.6(±0.71) ng/L range, thus accounting for 26.1-38.0% of the Σ16PAHs. Fluorene, phenanthrene, fluoranthene and pyrene were also present in all the analyzed samples, with concentration levels in the 0.94(±0.02)-3.32(±0.11), 1.73(±0.04)-8.83(±0.12), 1.97(±0.15)-9.88(±0.09) and 1.68(±0.03)-6.01(±0.45) ranges, respectively. As shown in the table, low-molecular weight PAHs accounted for more than 85% of the Σ16PAHs, in accordance with previously reported findings (Arellano et al., 2018, 2014; Gabrieli et al., 2010). The prevalence of three- and four-rings PAHs in high altitude snowpacks have been reported in Europe, North America and Asia (Arellano et al., 2018). This behavior can be explained taking into account that snow is considered a very efficient scavenger of PAHs present in the atmosphere both in the gas phase and absorbed onto particulate matter. Pollutants can be transported to high elevation, where the temperature drop enhances the condensation of these compounds and the particulate matter acts as core for the nucleation of aqueous crystals, thus entrapping PAHs in the snowflakes. In addition, the porous structure and high surface/volume ratio of snowflakes increase the scavenging capabilities, capturing aerosol particles and gaseous hydrophobic molecules (Gabrieli et al., 2010). As reported in **Table 4**, the highest concentration of the Σ16PAHs was detected in Sample 6: these findings could be related to the presence of anthropic activities due to the presence of tourists. Finally, the concentrations obtained by applying the developed method were compared with those assessed by using the

EPA8272 method, properly adapted for the detection of high molecular weight PAHs. Non-significant differences between the results were achieved for the majority of the compounds (**Tables S3** and **S4**). Since the LOQs of the reference method were higher than those presented in this study for most of the investigated PAHs, the quantitation of the analytes present at sub-ng/L level could not be feasible by using the reference method. However, the matching of all the quantifiable concentrations proved the reliability of the developed method.

4. Conclusions

In the present study, a new SPME coating based on the BenzoQxCav receptor was developed and tested for the SPME-GC-MS determination of PAHs at trace levels. The developed material provided excellent performance in terms of extraction capabilities and selectivity, thus resulting in very low detection and quantitation limits. The high affinity of the coating towards PAHs was related to the complexation of the target analytes inside the receptor *via* π-π and CH-π interactions. The enhanced selectivity was possible due to the presence of a 8.3 Å deep cavity, suitable for hosting bulky guests such as PAHs, with a very high degree of fitting. The extraction capabilities of the selective coating were not influenced by the presence of other potential interfering aromatic compounds also when present at concentration levels 14 times higher than those of the investigated PAHs.

The analysis of snow samples from Antarctica and Alps confirmed the widespread presence of low-molecular weight PAHs.

The major features of the proposed method are related to the high sensitivity and selectivity, the reduced sample handling and the possibility of matching the requirements of green analytical chemistry. Therefore, it will be used in future studies to screen the presence of PAHs in different environment to assess the anthropogenic impact and to study pollutant transport mechanisms.

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