MicroFlow LC: A Viable Solution for PFAS Analysis

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Abstract

Combining microflow chromatography with a triple quadrupole system provides extraordinary sensitivity for PFAS compounds in drinking water and soils. We describe the configuration and provide data on robustness and sensitivity. Sensitivity gains over high flow chromatography are demonstrated.

Introduction

Per- and polyfluorinated alkyl substances (PFAS) are a group of approximately 5,000 manmade chemicals that contain carbon-fluorine (C-F) monomers, most of which are highly stable and resistant to degradation [1,2]. These C-F bonds repel both oil and water in nature, making PFAS useful for many applications, including personal care products, food packaging, textiles, and fire-fighting foam. However, due to the abundance and strength of the C-F bonds, natural degradation of these compounds in the environment is extremely difficult, making them highly persistent [2,3]. The overwhelming presence of PFAS in drinking water systems and humans motivated the United States Environmental Protection Agency (U.S. EPA) to monitor 14 PFAS compounds, including perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS), in drinking water in Method 537 [4]. This method has been updated to EPA Method 537.1 and EPA Method 533.

The U.S. EPA advisory level of PFOA and PFOS combined is 70 ng/L in drinking water. However, some studies have suggested this level might be 100-fold too high [5]. This has influenced some states, like Vermont, to impose or offer lower acceptable limits. In 2016, Vermont adopted an advisory level of 20 ng/L for PFOA and PFOS combined, with other states like Minnesota, New Jersey, and Michigan following suit with their own levels.

As the suggested concentration limits continue to decrease and water system operators take appropriate steps to remove PFAS, more sensitive and robust analytical methods are needed. For routine environmental testing laboratories, preparing and analysing samples with a fast turnaround of results is crucial. The added demand pushes the need for new methods and technologies that will improve workflows; workflows need to include efficient and effective means to extract PFAS and remove matrix components during sample preparation and increase accuracy and precision. Challenges arise due to the compounds' ubiquitous and persistent nature, which affects the ability to push instrument detection limits that are impeded by high background and baseline.

Table 1: MFLC gradient for microflow EPA Method 537 analysis

Time (min)	% Mobile Phase A	% Mobile Phase B
0	98	2
1.2	45	55
7	1	99
8.5	0	100
8.6	98	2

Microflow liquid chromatography

Modern analytical instruments continue to experience the demand for increased sensitivity while simultaneously consuming fewer resources (i.e. solvents) to maintain high efficiency and a competitive cost per analysis. Fortunately, access to techniques such as liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) has increased. LC offers ideal separation, identification, and detection in complex samples.

In recent years, innovation in microflow LC systems coupled with mass spectrometry (MS) has pushed this envelope further, enabling labs to do more: achieve higher sensitivity with an even lower limit of quantification (LOQ), achieve higher throughput and better robustness in their analyses, and, more importantly, do it in less time. In addition, microflow LC utilises significantly less solvent and less analytical sample without sacrificing any performance features. This study presents a microflow method for analysing the EPA Method 537 on the SCIEX Triple Quad 6500+ system coupled with an OptiFlow Turbo V ion source on an M5 MicroLC system.

Since EPA Method 537 requires samples to be prepared in a relatively high proportion of organic solvent (i.e., 96:4 methanol: water, vol/vol), breakthrough and poor peak shape of the early eluting, more polar PFAS compounds analytes are common. This is due to the fact the method uses reversed phase liquid chromatography, even at low

injection volume. Therefore, this study utilised an online mixing strategy - the analytical conduit adapter (AnaCondA) - which effectively reduces the solvent strength at the front of the LC column. The effect of using the AnaCondA is reduced peak shape distortion and peak splitting in microflow chromatography due to the lower flow rate and smaller column diameter. In addition, it allows for higher injection volumes, which ultimately lower detection limits.

The same approach is also applicable to EPA Method 537.1, since it requires the same sample solvent composition as Method 537. EPA Method 533 permits a 'weaker' sample solvent composition (i.e., 80:20 methanol: water, vol/vol), which may lessen the need for the AnaCondA, although this has not been thoroughly investigated.

Materials and methods

Sample preparation: Sample preparation and data processing were carried out according to EPA Method 537. An additional 1/10 dilution was then performed. A total of 20 samples were extracted out of a variety of matrices, including drinking water, groundwater, wastewater, and soil extracts. The internal standards (ISTD) used were $^{13}C_2$-PFOA, $^{13}C_4$-PFOS, and d_3-N-methyl perfluorooctane sulfonamidoacetate (d_3-NMeFOSAA). The surrogates used were $^{13}C_2$-perfluorohexanoic acid ($^{13}C_2$-PFHxA), d_5-N-ethyl perfluorooctane sulfonamidoacetate (d_5-NEtFOSAA), and $^{13}C_2$-perfluorodecanoic acid ($^{13}C_2$-PFDA). The complete sample set, including calibration and quality control samples, was run on 3 separate days.

Chromatography: The microflow analysis was performed using an M5 MicroLC system at a flowrate of 10 µL/min. A Gemini C18 3 µm, 100 x 0.3 mm column (Phenomenex) was used. This column uses the identical stationary phase, but smaller internal diameter as the high-flow method [5]. Mobile phases A and B were Milli-Q water with 10 mM ammonium acetate and J.T.Baker Ultra LC-MS grade methanol with 10 mM ammonium acetate, respectively (Table 1).

A novel online AnaCondA mixer was placed upstream of the analytical column (Figure 1). This approach works by increasing the Reynolds number (Equation 1) by increasing the diameter of tubing after the sample loop, thus promoting turbulence and creating more mixing. This works on the same principle as the Performance Optimizing Injection Sequence (POISe) approach outlined in Sanchez et al. 2012 but eliminates the need to make an additional injection of weak solvent [7]. In the AnaCondA approach, the weaker solvent (mobile phase A) is being mixed with the sample due to the trainstion from the low velcocity zone caused by the AnaCondA's larger diameter to the high velocity zone caused by the small diameter of the microflow tubing.

$$Re = \frac{\rho V D}{\mu}$$

Re = Reynolds Number
ρ = Density of mobile phase
V = Velocity
D = Diameter of anaconda
µ = Dynamic viscosity of mobile phase

Equation 1. Reynolds number equation

Typically, the high injection solvent strength required by EPA Method 537 causes excessive breakthrough and peak splitting, even with a 1 µL injection volume (Figure 2a). To prevent this from occurring, online mixing was promoted using an AnaCondA with a wide internal diameter (ID) of 0.5 mm and length of 5 cm after the sample loop (Figure 1). In addition to the AnaCondA, a faster sample injection speed was performed to increase the mixing turbulence. This allowed the injection volume to range between 1 and 10 µL without breakthrough or peak splitting (Figure 2b). The data shown in this application note were generated using a 4 µL injection volume to represent a traditionally monitored concentration range.

Mass spectrometry: The sample was injected into the SCIEX Triple Quad 6500+ system equipped with a OptiFlow Turbo V ion source that was designed specifically for lower flow rates. The optimised source conditions can be found in Table 2.

All analytes were monitored in multiple reaction monitoring (MRM) scan mode in

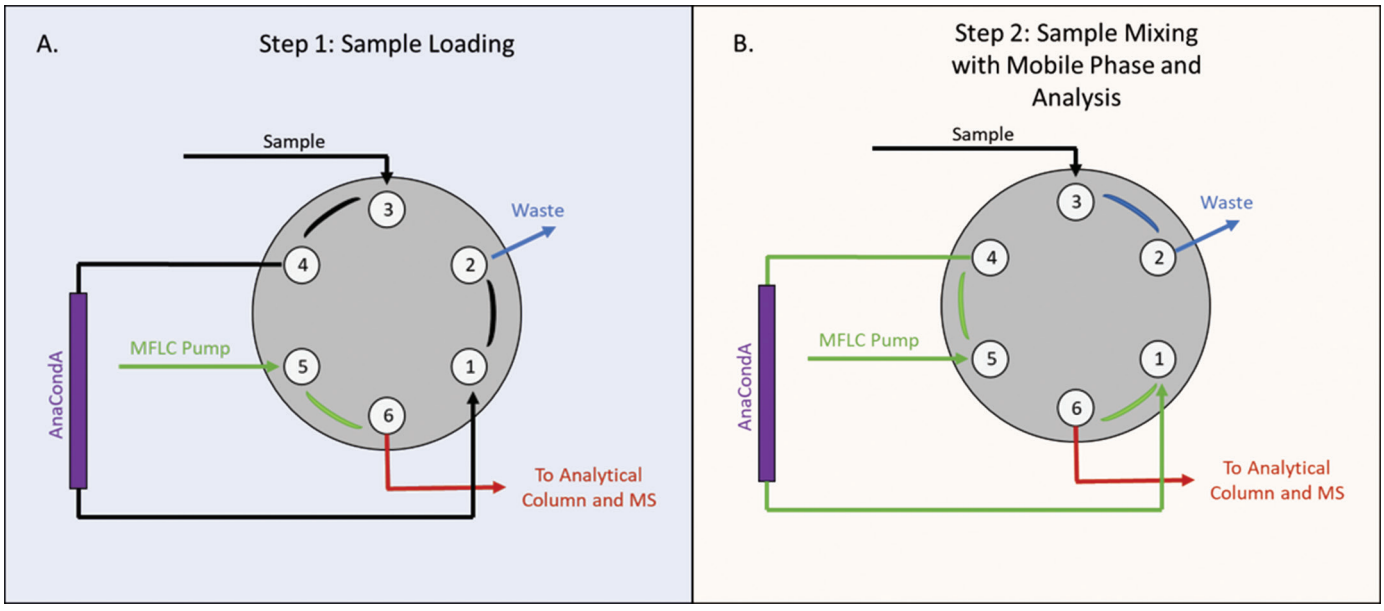

Figure 1. Microflow LC setup with analytical conduit adapter (AnaCondA) for sample mixing. A. Step 1 represents when the sample is being loaded into the AnaCondA while the microflow liquid chromatography (MFLC) pump flow is going to waste B. Step 2 represents the mixing phase where MFLC pump flow is being introduced to the AnaCondA mixing mobile phase A composed of Milli-Q water with 10 mM ammonium acetate with the injected sample effectively diluting the methanol concentration.

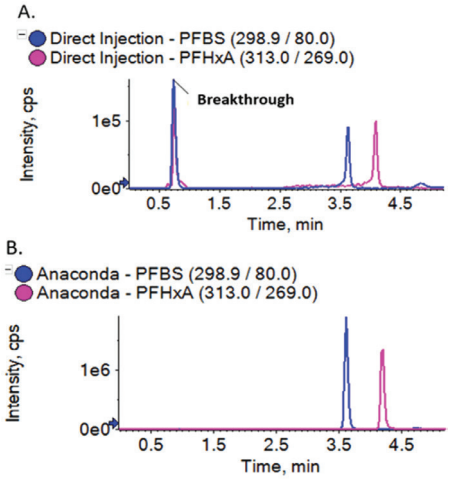

Figure 2. Advantage of using the online analytical conduit adapter mixer for microflow PFAS analysis. (Top) Example chromatograms of PFBS and PFHxA using direct injection without mixing with microflow chromatography. (Bottom) Same microflow chromatography with online mixing using the AnaCondA.

negative polarity. The Scheduled MRM algorithm was used to monitor compounds during a 60 second expected retention time window to maximise dwell times and optimise the cycle time of the method.

Data processing: Results were processed in SCIEX OS software 1.7. Peak asymmetry and ion ratios were automatically calculated using custom columns. All calibration curves had a 1/x concentration weighting and were forced through the intercept as specified in EPA Method 537.

Parameter	% Mobile Phase A
Curtain Gas (CUR)	20 psi
Ionspray Voltage (IS)	-4500 V
Heater Temperature (TEM)	300ºC
Gas 1	15 psi
Gas 2	60 psi

Discussion

Robustness and reproducibility: Microflow LC has been widely used in pharma and biopharma applications but has infrequently been used in environmental applications. To ensure ruggedness of both the method and analysis, calibration curves were generated and drinking water and soil samples were acquired in triplicate over 3 separate days. To evaluate whether suppression was occurring throughout the calibration curve process, the ISTD areas were plotted over the 3-day run for all calibration and quality control samples (Figure 3, top). The mean ISTD area was calculated and all collected data points fell within ±20%, suggesting no major suppression was occurring. The surrogate concentrations were also plotted over the 3-day run and were within the acceptable ±30% outlined in EPA Method 537 (Figure 3, bottom).

Sensitivity: The result indicates a significant gain in sensitivity (Figure 4). The 9 or 10-point calibration curve exhibited good accuracy within ±30% of the expected values for all points, accuracy within ±50% for the lowest calibrator, and R^2 coefficients of >0.990 (Table 4). The lower limit of quantification (LLOQ) varied between 1 and 5 parts per trillion (ppt) in vial, equating to 0.04 and 0.2 ppt in the sample before extraction (Table 3; Figure 5). If further sensitivity was needed, a larger injection volume (up to 2.5x larger) could be performed.

The sensitivity between the presented microflow LC method and traditional flow method [5] using a 4 µL injection was compared. This comparison was made by dividing the signal to noise (S/N) for the compound using the microflow LC method by the S/N of the compound using the traditional flow method. This ratio was measured at the lowest point of the calibration curve in the traditional flow data, given that the microflow LLOQ was significantly lower. When comparing sensitivity gains from the current microflow method to traditional flow, all PFAS compounds showed improved sensitivity from the smaller flow rates. The exact change in peak signal intensity varied across the panel largely due to individual analyte properties (data not shown). However, the sensitivity gains ranged from 2.2 for PFOS to 24.2 for PFTeDA.

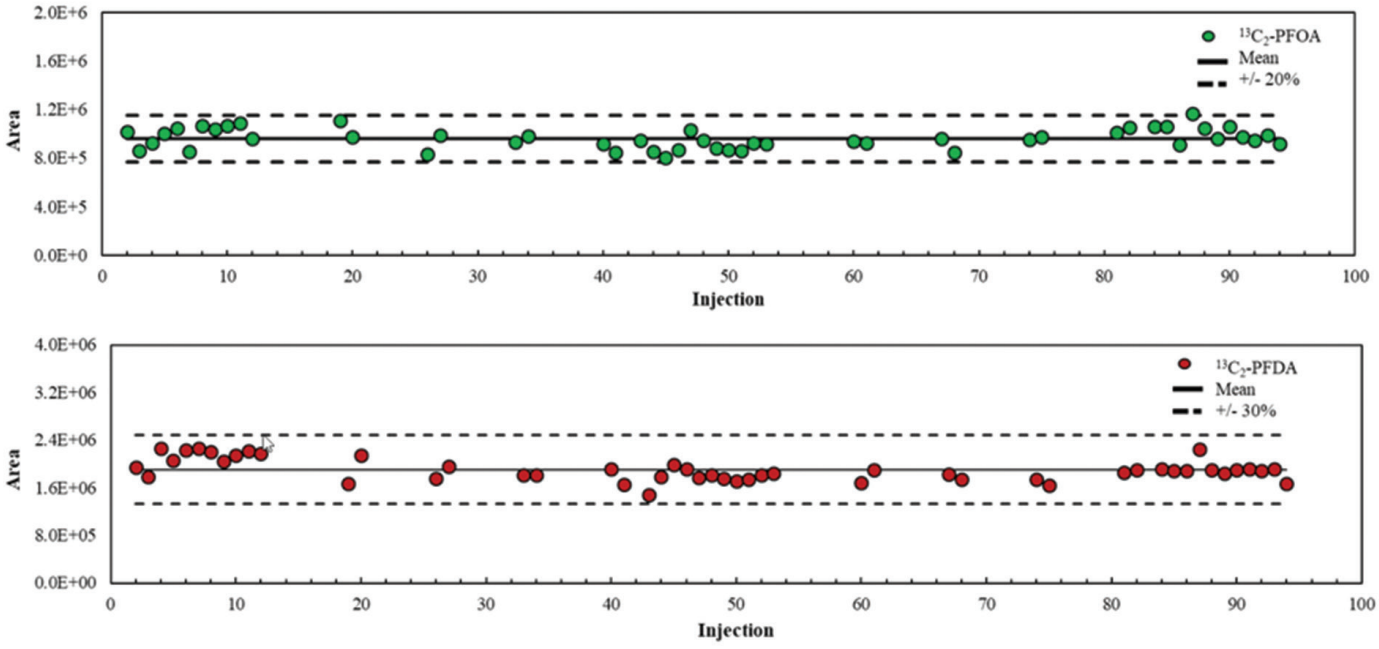

Figure 3. Reproducibility of data. $^{13}C_2$-PFOA (used as an internal standard, top) and $^{13}C_2$-PFDA (used as a surrogate, bottom) in the analysis were plotted for all standards, QCs, and blanks.

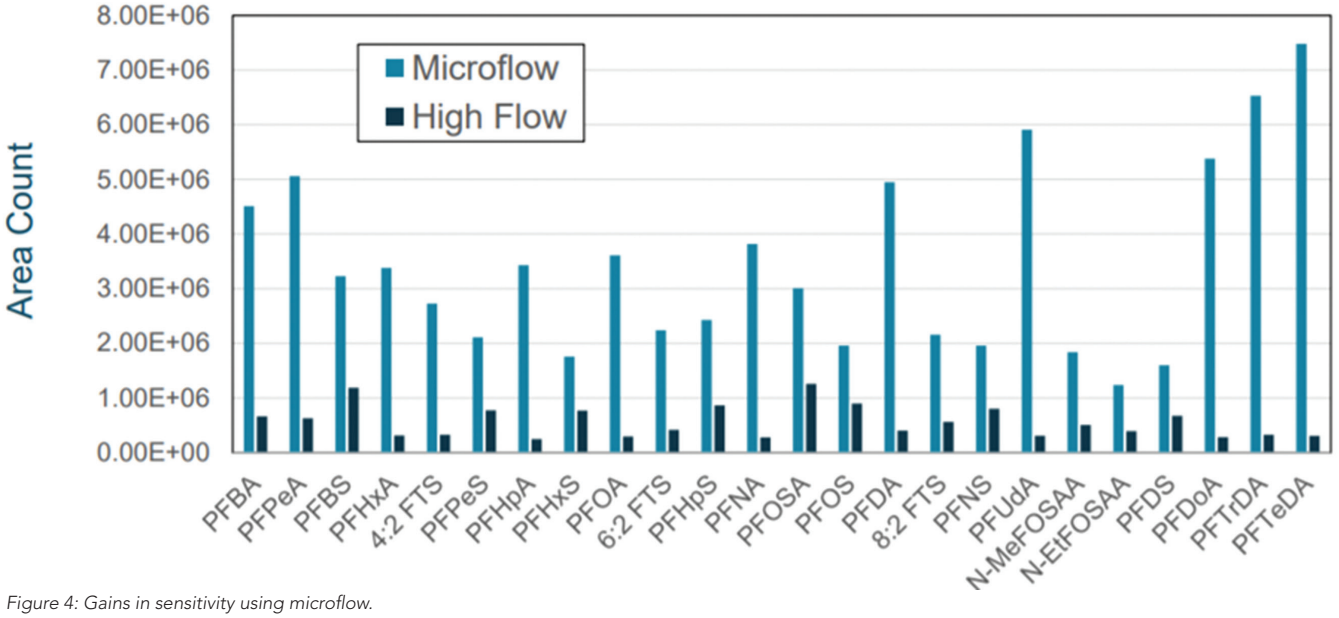

Figure 4: Gains in sensitivity using microflow.

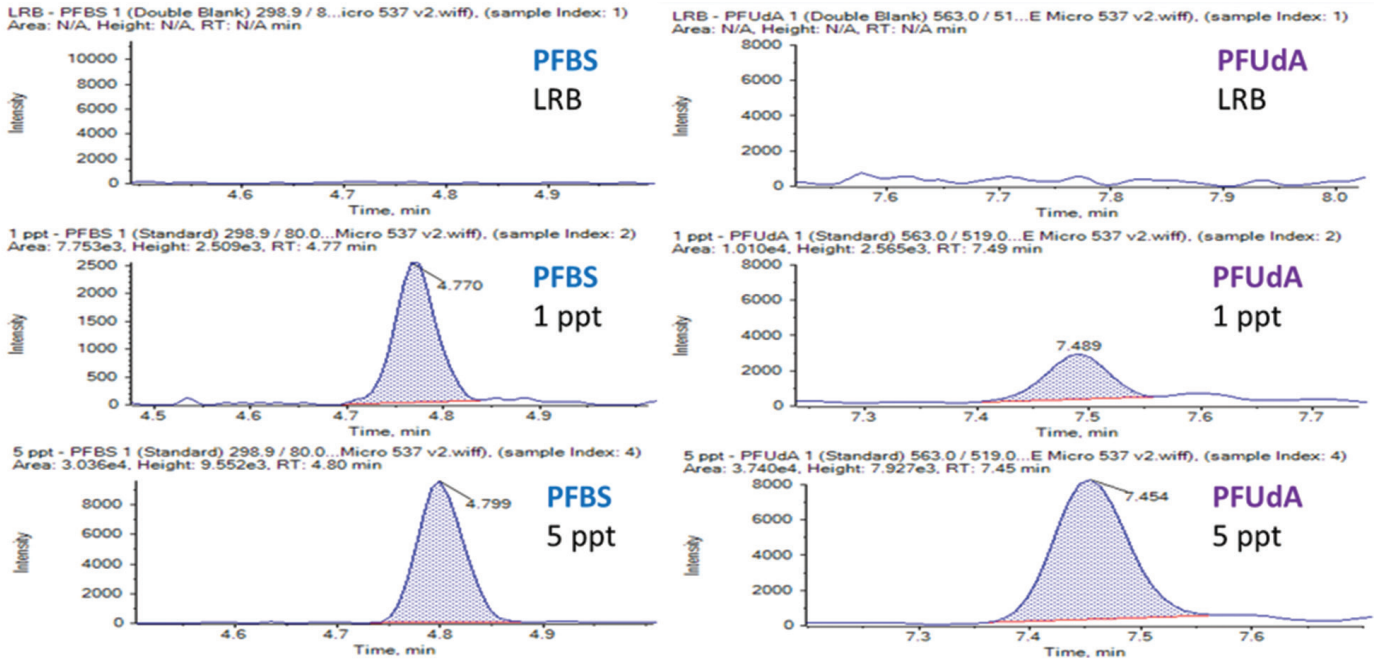

Figure 5. Example LLOQ chromatograms. PFBS (left column) and PFUdA (right column) showing a laboratory reagent blank (LRB), 1 ppt, and 5 ppt standards.

Table 3: The LOQ of EPA 537 PFAS components in vial and in the extracted sample.

Component name	LLOQ	ULOQ	R^2	Sample LLOQ (ppt)
PFBS	1	2500	0.998	0.04
PFHxA	5	2500	0.996	0.2
PFHpA	5	2500	0.998	0.2
PFPeS	1	2500	0.998	0.04
PFHxS	5	2500	0.998	0.2
PFOA	1	2500	0.997	0.04
PFNA	1	2500	0.997	0.04
PFOS	1	2500	0.999	0.04
PFDA	1	2500	0.999	0.04
N-MeFOSAA	5	2500	0.996	0.2
N-EtFOSAA	5	2500	0.992	0.2
PFUdA	1	2500	0.999	0.04
PFDoA	1	2500	0.998	0.04
PFTeDA	5	2500	0.996	0.2

Conclusions

A sensitive and robust method was developed for microflow analysis of the analytes in EPA Method 537. The assay showed reproducibility of internal standards, surrogates, and calculated concentrations of unknown environmental samples over multiple days. The increase in sensitivity in this study enabled LLOQs of 1-5 ppt for EPA Method 537 with a 4 µL injection volume. A larger injection volume, enabled by the AnaCondA mixing approach, would allow for even lower LLOQs, if necessary. This method has been updated to support EPA Method 537.1 and EPA Method 533.

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GC-MS Air Analyser for Very Low Concentration Determination of VOCs in Indoor and Ambient Air to Method TO-17

A new instrument for the automated, in situ, determination of airborne volatile organic compounds (VOCs) using the OPTIC-4 Multimode Inlet for gas chromatography has been introduced by **GL Sciences BV** and ChromaVision. The technology features a sorbent tube placed as an injection port liner which can be repeatedly used to collect samples of air, with the trapped analytes being subsequently desorbed onto a capillary gas chromatography (GC) column without the use of intermediate cryogenic refocusing. As the system has no need for any liquid nitrogen or CO_2, there is no need for adsorption/desorption tubes. It is possible to set a continuous run so that for on-line analysis the system can run 24-7.

Using a multi positioning valve, the system can select between standards or taking sample directly from the outside via, e.g., a probe on the roof of a mobile lab. Sampling is done during a fixed time with a constant flow controlled with a mass flow controller. Once the sampling period is finished, the carrier gas flow via the injector port is re-established. Following this, the injection port is heated to desorb the analytes from the injection port liner for transfer to the GC capillary column. In parallel with heating the injection port liner, the GC–MS analysis is started.

A home-made mixture of 63 compounds (comparable to the TO-17 mixture) is used for evaluation of the Air Analyser. Via a smart valving system, sample is loaded from bottom-to-top on the OPTIC Air Liner, being located inside the injection port at a temperature of 25°C The injection port is cooled with compressed air. Sampling is done during three minutes at 75 ml/min (total sample volume = 225 ml). After sampling, the carrier gas is re-routed through the injection port from top-to-bottom. Following this, the injection port is heated to 270°C, desorbing the trapped compounds to the capillary column using spitless transfer. The resulting chromatogram starts with n-Propane and ends with Naphthalene. Since the sample trap is in the injection port, no additional cryogenic focusing is required at the head of the capillary column.

More information online: ilmt.co/PL/qa9m