



Application Note 263

Improving extraction efficiency of SPME on soil samples by using SPME–trap and SPME–trap with enrichment

Solid-phase microextraction (SPME) is a commonly used sampling technique for the profiling of volatile organic compounds (VOCs) from soil; however, the technique is limited by the amount of phase available. The introduction of SPME–trap and SPME–trap with enrichment is a step change towards improving sample extraction and reaching lower detection limits while strengthening confidence in compound identification. This application note demonstrates the possibility to extend the number of identified peaks from a complex soil matrix. Utilising SPME–trap with enrichment, the number of compounds identified were increased from 544 (SPME–trap) to 656. SPME–trap with enrichment is an extension of SPME on the Markes Centri® sample extraction and enrichment platform.

Introduction

The study of soils is fundamental for a wide range of ecosystem services (e.g., nutrient cycling, water purification, food provisioning and climate regulation). The health of soils affects the whole earth system,¹ so it is essential that soil health is monitored to ensure these ecosystems are well maintained. Degradation of soil health is typically caused by anthropogenic sources (industrial pollution, agriculture, improper disposal of waste) that have an adverse impact on the global economy, with an estimated annual cost of \$231 billion. In the UK and USA alone, these annual costs are estimated to be \$2 billion and \$22 billion, respectively.²

Robust biological quality indicators for monitoring soil health that can be widely adopted across the agricultural sector have remained elusive.³ VOC profiles are used as non-destructive fingerprints allowing the monitoring of organisms and enabling an understanding of bi- and multi-trophic interactions between microorganisms, plants and invertebrates in the soil.⁴ A diverse range of VOCs, including carboxylic acids, alcohols, terpenes, amines, esters and alkenes, can be found in soils.⁵ Detecting such a wide array of compounds across a large concentration range from a complex matrix is a challenging task.

Typically, headspace solid-phase microextraction (HS-SPME) has been the method of choice for sample preparation.⁶ This method can be limited in its sensitivity by the amount of phase available on a SPME fiber (~0.5 µL); this leads to competition between analytes for absorption onto the phase. In this study, the sensitivity of SPME is increased by using SPME–trap and SPME–trap with enrichment to analyse VOCs from a soil sample.

What is SPME–trap with enrichment?

SPME is a well-established technique for the sampling of VOCs from different sample matrices. It is a fast and simple technique that does not require the use of solvents. SPME can be used to extract volatiles from a headspace or by immersive sampling from a liquid sample. Detection limits for SPME are reported to reach parts per trillion (ppt) levels for certain compounds. However, combining SPME with a focusing trap and extracting multiple times extends SPME to reach even lower detection limits by effectively increasing the amount of absorption phase available, while maintaining the versatility of the SPME fiber.

The introduction of the novel sample introduction technique SPME–trap with enrichment on the Centri platform makes it possible to desorb the saturated phase to the focusing trap, and condition and re-introduce the SPME fiber to the sample (Figure 1). This process continuously depletes the headspace of the sample, ultimately eliminating the competition between analytes for absorption onto the phase often seen in SPME analysis.⁷

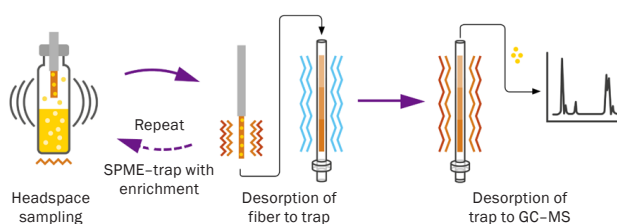


Figure 1: Three-step workflow for SPME–trap with enrichment.

Background to Centri and SPME

The **Centri** system from Markes International is the first GC–MS sample extraction and enrichment platform to offer high-sensitivity unattended sampling and preconcentration of VOCs and SVOCs in solid, liquid and gaseous samples.

Centri allows full automation of sampling using HiSorb high-capacity sorptive extraction, headspace, SPME, and tube-based thermal desorption.

Leading robotics and analyte-trapping technologies are used to improve sample throughput and maximise sensitivity for a range of applications.



In addition, Centri allows samples from any injection mode to be split and re-collected onto clean sorbent tubes, avoiding the need to repeat lengthy sample extraction procedures and improving security for valuable samples, amongst many other benefits.

The **SPME** and **SPME-trap** capabilities used in this study offer fast and effective sample extraction.

Sample extraction and transfer of the SPME fiber – either to the trap or directly to the GC system – can be automated.

For more on Centri and SPME, visit www.markes.com.

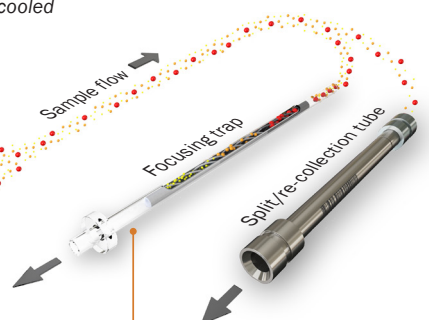
ANALYTE TRAPPING ON CENTRI (optional for headspace and SPME)

1 Sample focusing

Extracted analytes are swept into Centri's electrically-cooled focusing trap.

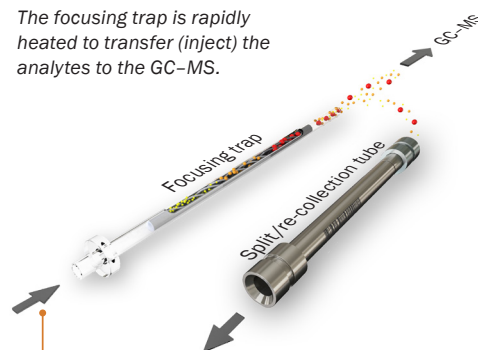
HiSorb high-capacity sorptive extraction
Headspace
SPME
Thermal desorption

Gas-phase internal standards can be introduced to the Centri trap in the carrier gas stream, as a check on the focusing and desorption processes.



2 Trap desorption

The focusing trap is rapidly heated to transfer (inject) the analytes to the GC–MS.



The carrier gas flow reverses during trap desorption to allow simultaneous analysis of compounds over a wide volatility range (VOCs and SVOCs).

Experimental

Sample:

A roadside soil sample was taken in the vicinity of Cardiff, UK (51°32'34"N, 3°23'17"W), and represents a typical soil exposed to vehicular activity. 2 g of the sample were weighed into a 20-mL headspace vial, which was then crimp-capped.

Sampling and preconcentration:

Instrument: Centri (Markes International)

Headspace SPME-trap:

Fiber: Multi-phase, 20 mm long, 50/30 µm d_f, DVB/CAR/PDMS (Supelco part no. 57299-U)
Position: 10 mm above the sample
SPME-trap: Extraction 95°C (1 h) SPME-phase exposure to headspace
SPME-trap with enrichment: Extraction 95°C (1 h) SPME-phase exposure to headspace (repeated three times)
Fiber desorption: 260°C (3 min)

Preconcentration:

Flow path: 180°C
 Focusing trap: 'Air Toxics' (part no. U-T15ATA-2S)
 Purge flow: 50 mL/min (1 min)
 Trap low: 20°C
 Trap high: 280°C (5 min)
 Trap heat: Max
 Split ratio: 5:1

GC-MS:

Column: MEGA®-624, 30 m × 0.25 mm × 1.4 µm
 Constant flow: Helium, 2.0 mL/min
 Oven program: 35°C (3 min), then 10°C/min to 100°C, then 20°C/min to 220°C (5 min)
 Transfer line: 230°C
 Ion source: 230°C
 Quad: 150°C
 Mass range: m/z 35–350

Software:

ChromSpace® (SepSolve Analytical)
 NIST Library (version 2.3, 2017)

Results and discussion

VOCs from a large variety of compound classes (including carboxylic acids, alcohols, terpenes, amines, esters and alkenes) were identified in the soil sample when using both SPME-trap and SPME-trap with enrichment (Figure 2). The chromatogram of the SPME-trap sample showed 544 distinct peaks when the data was processed using ChromSpace software. Comparing against the NIST Mass Spectral Library, 47 peaks could be assigned to spectra with a match factor of 850 or higher with the single-quad detector. The most prominent compounds identified in the soil sample were benzaldehyde, n-octanal, cyclosativene, calarene and 4,9-muroladiene.

While SPME-trap resulted in 544 peaks, SPME-trap with enrichment delivered a total of 656 peaks. 67 of these peaks could be identified with a match factor above 850 with the single-quad detector.

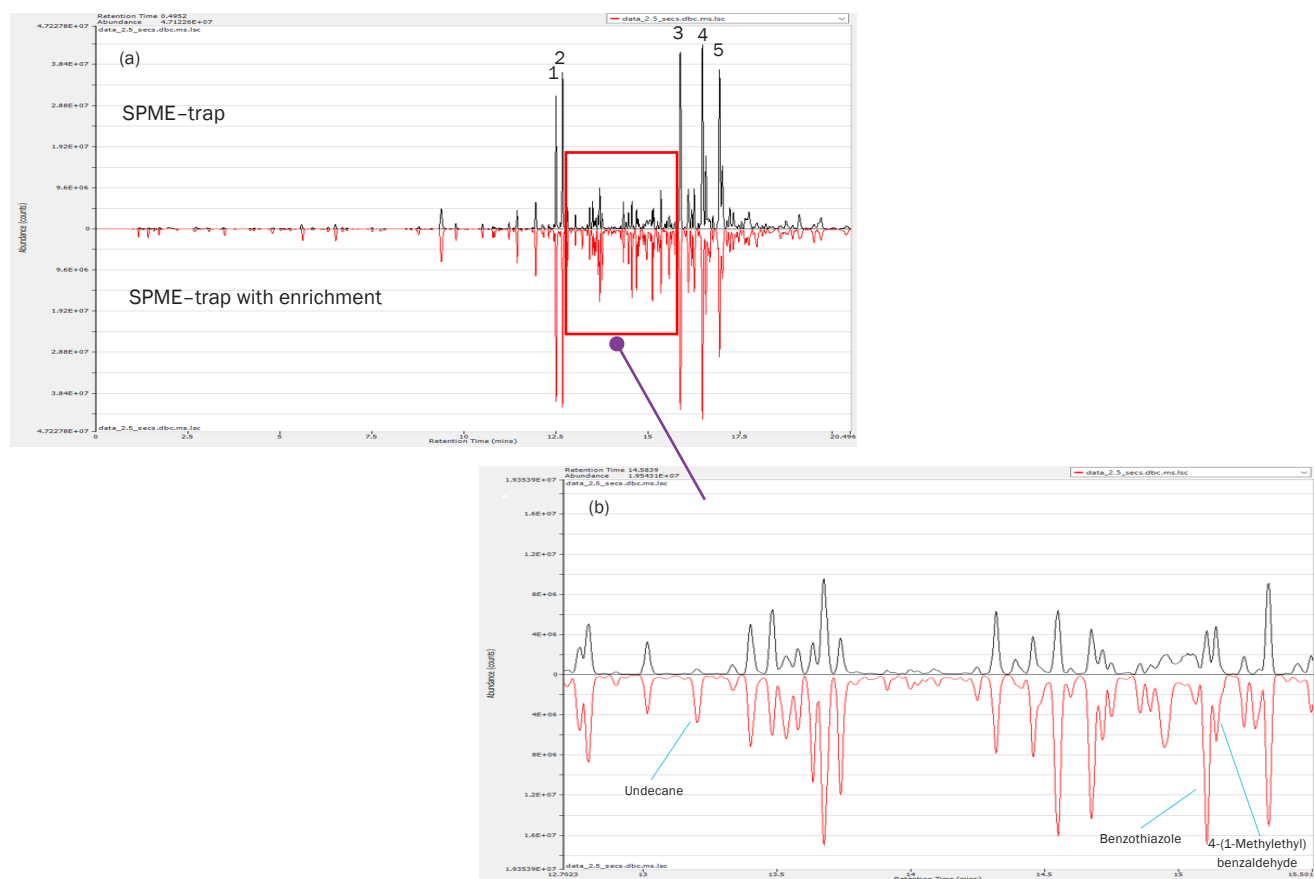


Figure 2: (a) Total VOC profile for a soil sample using SPME-trap and SPME-trap with enrichment. The most prominent compounds identified in the sample were (1) benzaldehyde, (2) n-octanal, (3) cyclosativene, (4) calarene and (5) 4,9-muroladiene. (b) The chromatogram between 12.7 and 15.5 mins is highlighted, detailing the increase in sensitivity from SPME-trap to SPME-trap with enrichment and indicating excellent peak shapes for the VOCs. Undecane, benzothiazole and 4-(1-methylethyl)benzaldehyde show a significant increase in response when extracted multiple times.

Relevance of VOCs to soil health

Many VOCs perform a biological function, for example transmitting information between microorganisms in soil. These bacteria-derived volatiles can have a positive effect on growth and metabolic parameters in microorganisms without physical attachment. So a healthy soil needs a functioning microbial community.⁸

Monitoring these VOCs has been a challenge, as they are usually present in very low concentrations. This has typically been overcome by cultivating the microorganisms individually by addition of a substrate to increase the relative concentration of volatiles.⁹ This, however, can distort the original profile of the soil sample by removing the microorganisms from their original environment and can result in misleading interpretations. By overcoming the limitation of the single SPME extraction, lower concentrations are now visible (Figure 3).

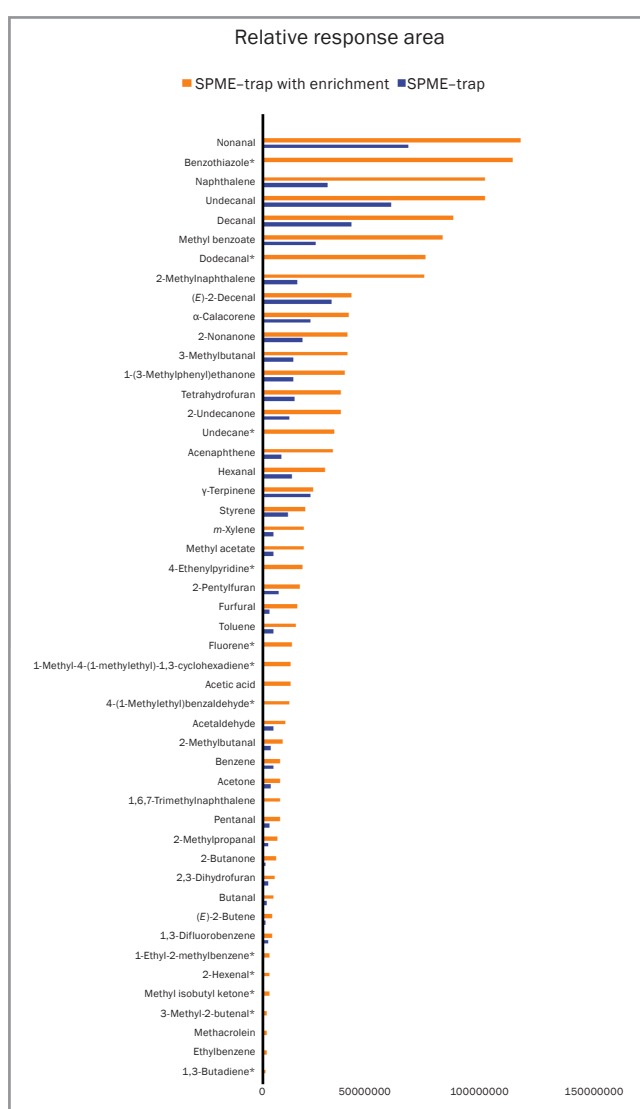


Figure 3: Comparison between the relative response areas obtained for compounds from a soil sample using SPME-trap *versus* SPME-trap with enrichment. Compounds were identified using the NIST Mass Spectral Library. *Some compounds detected using SPME-trap with enrichment that were not detected using SPME-trap.

The following are examples of substances found in soil that have a known biological function:⁸

- **Calarene:** Attracts other organisms to facilitate spore dispersal, resembling the function of volatile terpenes from the fruiting bodies of fungi.
- **Dodecanal:** At high concentrations, this defensive secretion inhibits the male copulatory response of the staphylinid beetle.
- **Nonanal:** Active against the phytopathogenic fungus *Sclerotinia sclerotiorum*.
- **Acetic acid:** Reduces heat-resistant spores and prevents spore formation.

Compounds such as acetaldehyde and propene have been particularly useful to determine soil health. They illustrate the agronomic effects of composts, mineral fertiliser and combinations of these on the chemical, biological and physiological properties of soil, ultimately giving an indication of soil health.¹⁰ SPME-trap with enrichment has given excellent peak shape and response after three extractions for these difficult early-eluting compounds (Figure 4).

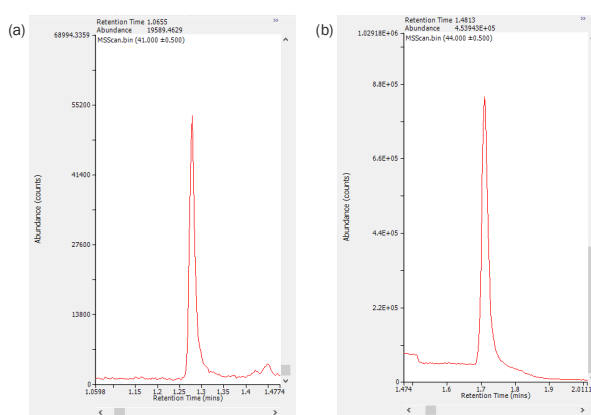


Figure 4: Extracted chromatogram for early-eluting (a) propene (m/z 41) and (b) acetaldehyde (m/z 44) sampled with SPME-trap with enrichment.

BTEX contaminates within soil

BTEX compounds (benzene, toluene, ethylbenzene and xylenes) are often detected around roads and other areas affected by emissions from the combustion of gasoline and diesel fuels. They are of concern because they are known carcinogens and endocrine disruptors.¹¹ The clear determination of BTEX compounds within soil is therefore an important factor for environmental monitoring. BTEX compounds have a high affinity with apolar matrices, such as soils, making them difficult to monitor, especially at lower levels.¹² Utilising the SPME-trap with enrichment, both BTEX compounds and microbial VOCs can be analysed in a single analysis.

Multiple extractions lead to much higher responses for BTEX compounds, ensuring clear identification and quantification (Figure 5). Three replicate samples were analysed for each mode (SPME-trap and SPME-trap with enrichment). The higher response shown in the results of SPME-trap with enrichment as well as the higher signal-to-noise ratio achieved highlight Centri's capability of decreasing detection limits therefore increasing confidence in the results.

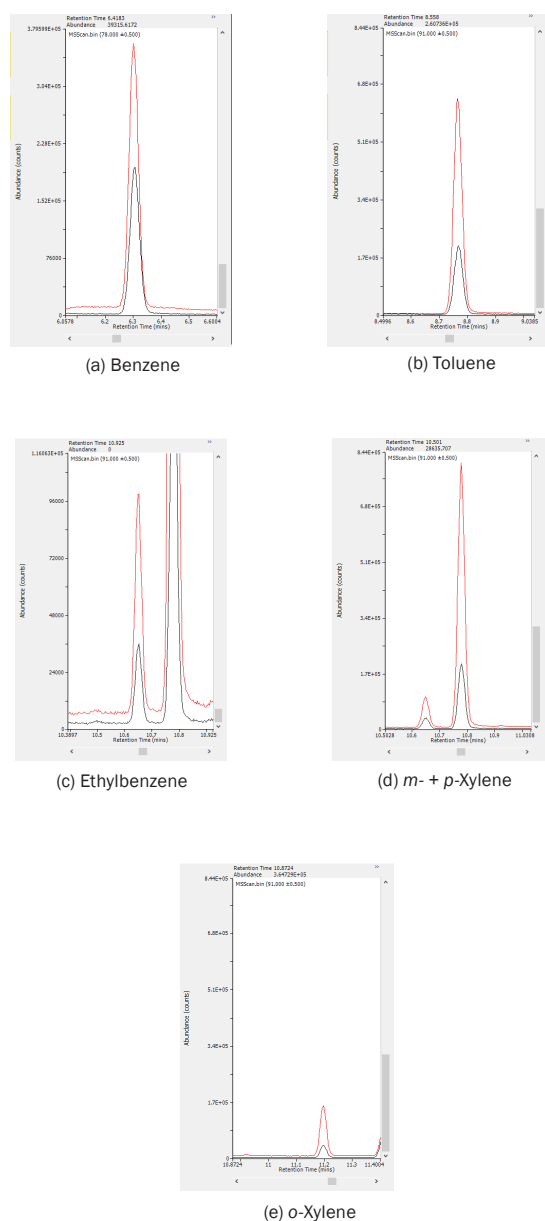


Figure 5: Extracted ion chromatograms for SPME-trap (lower peaks) and SPME-trap with enrichment (upper peaks), indicating the increase in response for the BTEX compounds benzene, toluene, ethylbenzene, m- + p-xylene and o-xylene in soils for three replicates of the soil sample.

Conclusions

This study has shown that the Centri sample extraction and enrichment platform is well-suited to extracting a large range of VOCs linked to biological functions directly from the headspace of soils using solid-phase microextraction. By utilising the trapping capabilities of the multi-bed sorbent trap, Centri can extend the sensitivity of standard SPME to identify a more comprehensive VOC profile.

When utilising the SPME-trap with enrichment mode on Centri, an additional 112 peaks were discovered from a soil sample, in addition to an increased response for contaminants (e.g., the BTEX compounds). Additional method development (e.g., optimising exposure time, extending GC run time and increasing the number of extractions) could result in a further increase in the number of compounds detected and an increase in sensitivity.

References

1. FAO, *Status of the World's Soil Resources. Main report [Policy Support and Governance]* Food and Agriculture Organization of the United Nations, 2015.
2. E. Nkonya, A. Mirzabaev and J. von Braun, Economics of Land Degradation and Improvement: An Introduction and Overview, in *Economics of Land Degradation and Improvement – A Global Assessment for Sustainable Development*, Springer International Publishing, 2016, 1–14, DOI: 10.1007/978-3-319-19168-3_1.
3. M. Schlöter, P. Nannipieri, S.J. Sørensen and J.D. van Elsas, Microbial indicators for soil quality, *Biol. Fertil. Soils*, 2018, 54: 1–10.
4. H. Insam and M.S.A. Seewald, Volatile organic compounds (VOCs) in soils, *Biol. Fertil. Soils*, 2010, 46: 199–213.
5. J. Peñuelas et al., Biogenic volatile emissions from the soil, *Plant, Cell Environ.*, 2014, 37: 1866–1891.
6. L.M. Dubois, P.H. Stefanuto, L. Heudt, J.F. Focant and K.A. Perrault, Characterizing decomposition odor from soil and adipocere samples at a death scene using HS-SPME-GC×GC-HRTOFMS, *Forensic Chem.*, 2018, 8: 11–20.
7. D.D. Roberts, P. Pollien and C. Milo, Solid-phase microextraction method development for headspace analysis of volatile flavor compounds, *J. Agric. Food Chem.*, 2000, 48: 2430–2437.
8. E. Amavizca et al., Enhanced performance of the microalga *Chlorella sorokiniana* remotely induced by the plant growth-promoting bacteria *Azospirillum brasilense* and *Bacillus pumilus*, *Sci. Rep.*, 2017, 7: 1–11.
9. O. Heinemeyer, H. Insam, E.A. Kaiser and G. Walenzik, Soil microbial biomass and respiration measurements: An automated technique based on infra-red gas analysis, *Plant Soil*, 1989, 116: 191–195.
10. M.S.A. Seewald et al., Substrate-induced volatile organic compound emissions from compost-amended soils, *Biol. Fertil. Soils*, 2010, 46: 371–382.

11. A.L. Bolden, C.F. Kwiatkowski and T. Colborn, New look at BTEX: Are ambient levels a problem? *Environ. Sci. Technol.*, 2015, 49: 5261–5276.
12. D. Orazbayeva, B. Kenessov, J.A. Koziel, D. Nassyrova and N.V. Lyabukhova, Quantification of BTEX in Soil by Headspace SPME–GC–MS Using Combined Standard Addition and Internal Standard Calibration, *Chromatographia*, 2017, 80: 1249–1256.

Centri® and ChromSpace® are trademarks of Markes International. MEGA® is a trademark of MEGA.

Applications were performed under the stated analytical conditions. Operation under different conditions, or with incompatible sample matrices, may impact the performance shown.