

The automation of vacuum assisted headspace solid phase microextraction (Vac-HS-SPME) and its application for analysis of volatiles in food

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Headspace solid phase microextraction (HS-SPME) is a well-established technique for the determination of volatiles in food. It is a solvent free sample preparation approach that can provide extraction and enrichment of analytes from a variety of matrices.

The rate-limiting step is the analyte transfer from the matrix, which means that for semi-volatile analytes methods can require long extraction times and/or high extraction temperatures to obtain the required sensitivity. For some food applications it is preferred to keep temperatures lower, to avoid formation of additional compounds, for example, due to Maillard reactions. Applying vacuum during HS-SPME has been shown to improve extraction kinetics for some semi-volatile sample components, resulting in higher extraction efficiencies and analyte sensitivity, with shorter sampling times and at milder sampling temperatures.

Publications to date have used manual application of vacuum, offline, followed by analysis on automated HS-SPME-GC-MS instrumentation. Automating the entire procedure will bring significant benefits, including speed, throughput, and the ability to run the system unattended.

Theoretical aspects

HS-SPME involves three partitions: sample matrix, sample headspace and fibre. This results in two concurrent thermodynamic processes and therefore two partition coefficients are involved in achieving a final equilibration. However, the majority of HS-SPME methods do not wait for the system to achieve the equilibrium state and typically extraction is performed for a defined period of time. In this scenario, the analytical performance depends more on the kinetics associated with the mass transfer of the analyte into the sample headspace.

Bias has been observed in HS-SPME methods towards the more volatile analytes, which have a higher affinity for the vapour phase therefore transition more readily into the headspace and subsequently onto the fibre. Common approaches employed to speed up the mass transfer kinetics for the semi-volatile components are the use of agitation or an increase in the temperature of extraction.

An additional or alternative way of accelerating extraction kinetics for semi-volatiles in HS-SPME is applying vacuum conditions, performing vacuum-assisted HS-SPME (Vac-HS-SPME).

The advantages of performing Vacuum Assisted Headspace solid phase microextraction (Vac-HS-SPME), compared to atmospheric pressure headspace solid phase microextraction (AP-HS-SPME) have been described extensively in the literature [1-7].

Figure 1 shows how Vac-HS-SPME gives faster extraction times at a given sampling temperature, or increased sensitivity at reduced sampling temperatures when compared to atmospheric pressure HS-SPME (AP-HS-SPME).

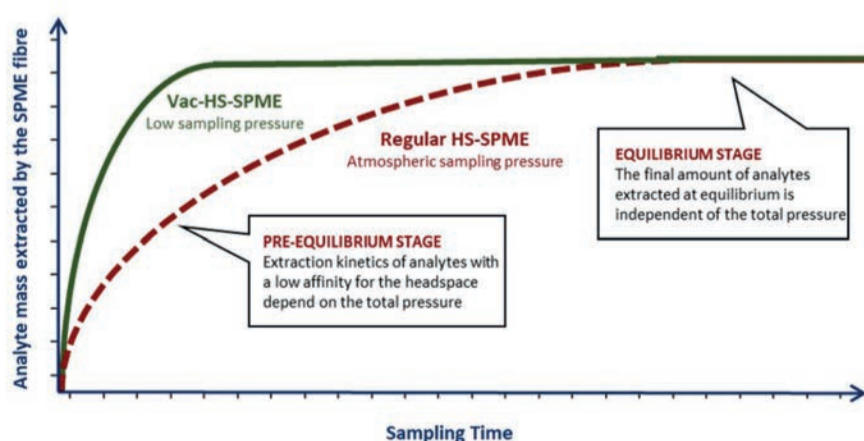


Figure 1: Extraction profile of compounds with low affinity for the headspace for regular atmospheric pressure HS-SPME and vacuum-assisted Vac-HS-SPME - kindly provided by E. Psillakis from publication *Anal. Chim. Acta*, 2017, 986, 12-24

For aqueous based samples, the effect of vacuum can be related to Henry's Law constant – relating to the partition between the water and gas phase. The reduced pressure will have the most impact in terms of improved extraction efficiencies for those compounds with a K_H value less than $1.6 \times 10^{-4} \text{ atm m}^3 \text{ mol}^{-1}$.

Experimental

Compared to standard HS-SPME, the only additional step required for Vac-HS-SPME is the application of vacuum to the sample container. When performed manually this can be achieved using a gastight syringe or a vacuum pump with tubing and Luer Lock attachment. The vacuum is maintained during sampling by the use of custom-made vial closures which can be used with standard SPME headspace vials (Figure 2).

In order to automate the evacuation process, a GERSTEL robotic multipurpose sampler was used with a selection of modules that were modified and tested to compare to the manual process. A Dual head Robotic/robotic Pro GERSTEL MPS was fitted with a Preparation Syringe Module (PSM) fitted with a 2.5ml syringe (no needle) and a SPME tool for sample extraction/introduction. Incubation was performed in a GERSTEL cooled Agitator. Evacuation was achieved using a modified purge tool connected to a vacuum pump with multiple headspace extraction (MHE) station. Details of this and how it is controlled in the GERSTEL Maestro software are detailed in Application note Element Lab Solutions [8].

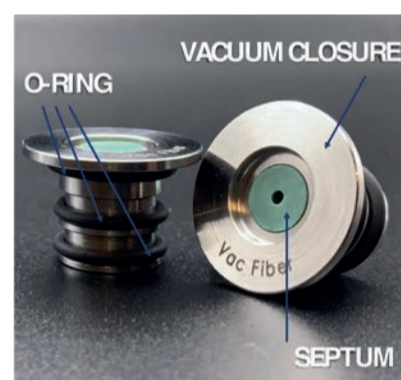


Figure 2: Extractech vacuum closures for Vac-HS-SPME.

Evaluation of food volatiles test mixture

Following testing of the automated evacuation procedure, experiments were conducted on a custom-made test mix to assess the performance compared to atmospheric pressure headspace SPME (AP-HS-SPME). Compounds chosen are detailed in Table 1.

Table 1: Analytes included in custom test mixture to assess performances on Vac-HS-SPME

#	NAME	CAS NUMBER	MOLECULAR WEIGHT [amu]	BOILING POINT [°C]	KH [atm m ³ mol ⁻¹]	RETENTION TIME [min]
1	2-pentanone	107-87-9	86.1	102	2.1E-04	2.41
2	2-pentanol	6032-29-7	88.1	115	2.1E-05	2.52
3	2,5-dimethylpyrazine	123-32-0	108.1	155	3.5E-06	6.35
4	Ethyl hexanoate	123-66-0	144.2	167	1.1E-03	8.41
5	2-nonanone	821-55-6	142.2	195	7.1E-04	10.72
6	Ethyl octanoate	106-32-1	172.2	208	1.6E-03	13.33
7	Whiskey Lactone	39212-23-2	156.2	94	Not available	16.18
8	Ethyl decanoate	110-38-3	200.3	241	3.2E-03	17.86
9	Delta Deca lactone	705-86-2	198.3	170	6.2E-06	19.85
10	Ethyl tetra decanoate	124-06-1	256.4	295	2.3E-02	25.64
11	Octadecane	593-45-3	254.4	316	1.9E-2	25.78

A bulk test mixture was prepared in water and analysed using the same methods for Vac-HS-SPME and AP-HS-SPME. Peak areas for the target analytes were recorded and compared (Figure 3).

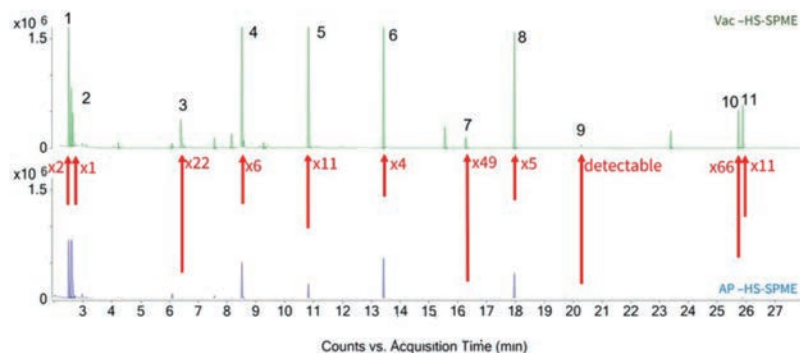


Figure 3: Chromatograms for AP-HS-SPME (bottom) and Vac-HS-SPME (top) for the investigated test mixture. The peak area increase factor for each of the targeted analytes as labelled in Table 1, are shown in red.

As predicted, the Vac-HS-SPME showed a considerable increase in peak area for the semi volatiles such as the whiskey lactone (x49) and the ethyl decanoate (x66). Particularly interesting was the trend observed for the Delta Deca lactone (9) which could not be detected at atmospheric pressure but was above LODs under vacuum conditions. It is worth highlighting that the SPME step was performed at low temperature (10°C) and for a short amount of time (10 min), which are atypical compared to standard HS-SPME approaches. This experiment was repeated with a 60-minute extraction time and this did give improved performance of AP-HS-SPME for some analytes, but for compounds such as the lactones, even with a 60 minute extraction at 10°C, they were only just detectable.

Summary/Conclusion

Vacuum-assisted headspace solid phase microextraction (Vac-HS-SPME) offers clear advantages for the analysis of semi-volatiles, in particular for applications such as food analysis where lower extraction temperatures and shorter extraction times can be beneficial in providing a more authentic sample profile.

Publications have previously demonstrated the benefits of vacuum - assisted headspace SPME for determination of volatiles in food [4,6,7,9,10]. This article describes the automation of the process using existing GERSTEL hardware and software, enabling a fully online solution for this approach. Initial experiments on a test mix have shown significant improvements in response for some semi-volatile analytes, such as lactones.

References

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