EXPLORING THE AUTOMATION OF VACUUM ASSISTED HEADSPACE SOLID PHASE MICROEXTRACTION (VAC-HS-SPME): PART I

APPLICATION NOTE AS-304

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Abstract

Headspace Solid Phase Microextraction (HS-SPME) is a well-established technique for the determination of volatiles by gas chromatography in a variety of matrices and applications.

However, due to analyte mass transfer being a rate-limiting step in the process, HS-SPME is intrinsically more effective in extracting volatile rather than semi-volatile analytes. Further, to facilitate the extraction of semi-volatile analytes, HS-SPME methods often require long extraction times and high extraction temperatures to speed up the kinetics of analyte transfer to the headspace However, this approach introduces the risk of sample decomposition or creation of new compounds such as with the Maillard reaction in food matrices, giving rise to artefacts within the chromatographic data.

Recently, an additional parameter has been proposed, to overcome this limitation: the application of vacuum. 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.

To date, Vacuum Assisted HS-SPME (Vac-HS-SPME) approaches have been carried out by applying the vacuum manually, offline, and then analysing the samples on the HS-SPME-GC platform. Automating this step will bring significant benefits to the operator including speed, throughput and the ability to run the system unattended. The evacuation can be added as part of the automated analysis method with no analyst 'touch time' involved.

This application note describes the development of an automated approach for Vac-HS-SPME using the GERSTEL Multipurpose Sampler (MPS)

INTRODUCTION

Solid-phase microextraction (SPME) is a widely adopted, solvent-free, sample preparation approach that combines sampling, extraction, enrichment, and sample introduction into one step.The SPME fibre-format technology remains the most commonly used, where a fused-silica rod coated with a limited amount of extracting phase is exposed for a predetermined time directly within the sample matrix or more commonly to the sample headspace (HS-SPME).

HS-SPME is, in fact, a three-phase system: sample matrix, sample headspace and extracting fiber. 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 with respect to both thermodynamic processes. Instead, the 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.

Mass transfer limitations are the reason for the often-observed bias in HS-SPME 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 tactics to speed up the mass transfer kinetics for the semi-volatile component are implementing agitation of the sample, maximising sample/headspace interface area or applying heating.

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).

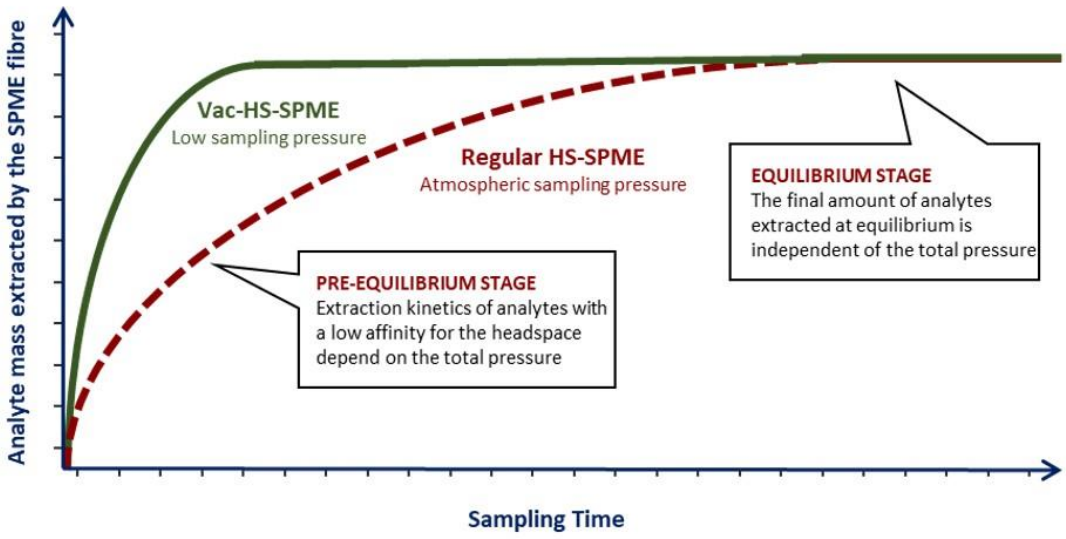


Figure 1: Extraction profile of compounds with low affinity for the headspace for regular atmospheric pressure HS-SPME and vacuum-assisted Vac-HS-SPME - kindly provided by E. Psillakis from publication Anal. Chim. Acta, 2017, 986, 12-24

The effect of vacuum on the sample/headspace system and consequently on the overall extraction kinetics can be predicted using a criterion based on Henry's Law Constant [2]. For aqueous-based samples, the effect is related to the partition between the water and the gas phase – so using K_H, we can predict those analytes for which the use of reduced pressure will have the most impact. For low K_H (less than 1.6 x 10-4 atm m^3 mol^{-1}) vacuum will improve extraction efficiencies when compared to AP- HS-SPME as longer equilibration times or higher temperatures are required. Alternatively, those analytes with high K_H values, similar extraction efficiencies will be observed between AP-HS-SPME and Vac-HS-SPME.

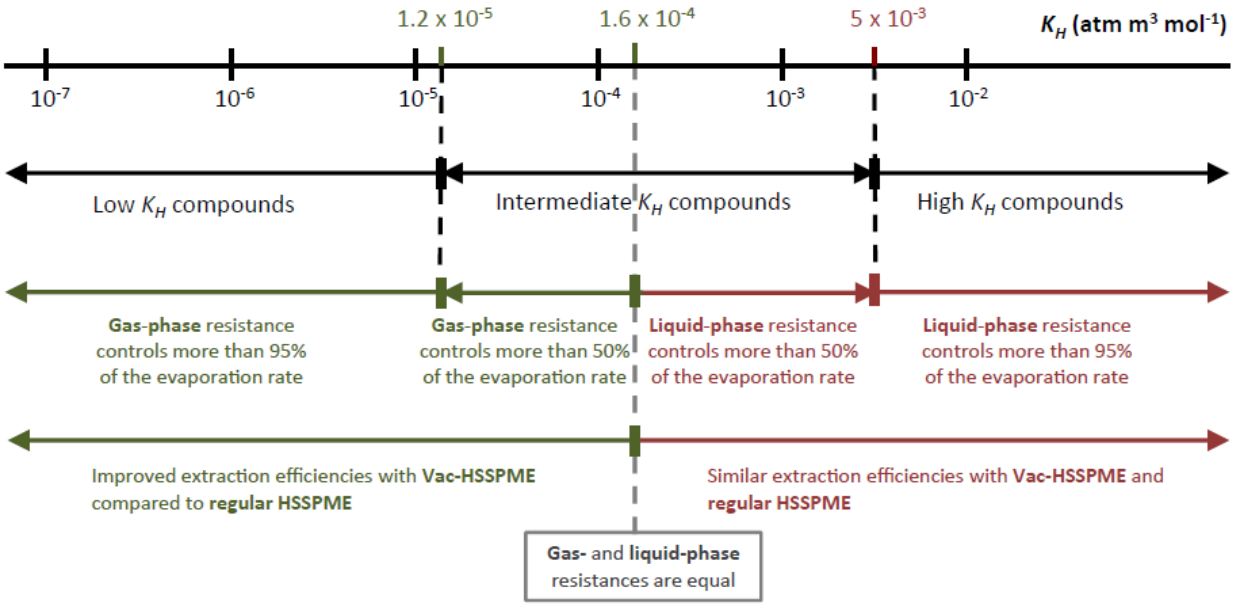


Figure 2: Prediction of vacuum effect for Vac-HS-SPME of water-based samples using Henry's law constants as the discerning criterion, kindly provided by E. Psillakis from publication Anal. Chim. Acta, 2017, 986, 12-24

Vacuum-assisted HS-SPME methods use identical analytical instrumentation and settings of standard methods - the only extra step needed is the removal of air from the sample container prior to SPME. Air evacuation is usually performed manually either using a gastight syringe to withdraw the volume of air from the sample vial or using a vacuum pump fitted with a connecting tubing with a Luer lock attachment to pierce the septum. A key component required to accommodate air evacuation and retain the vacuum within the sample vial during analysis is the use of custom-made vial closures which ensure a gastight seal on standard headspace vials.

This approach is normally performed offline, prior to analysis, hence automation of the process would be significantly beneficial in reducing analyst touch time and offer a seamless online solution. This work was dedicated to the development and testing of the automation of the air evacuation process using the GERSTEL Multipurpose Sampler and a selection of dedicated modules to perform the required tasks. The automated solutions were then tested to compare performances with the manual approach and evaluate robustness.

EXPERIMENTAL

INSTRUMENTATION

Automation

- GERSTEL MultiPurpose Sampler (MPS) Dual Head Robotic/Robotic Pro.
- *TOOLS:* Preparation Syringe Module (PSM) fitted with 2.5 mL syringe, no needle for the evacuation step and SPME tool for the HS-SPME step
- *MODULES:* Sample Storage on R60 Rack for 20mL vials, Evacuation on a MHE Station using a modified purge tool connected to a Vario Vacuum Pump PC3001, Incubation using a GERSTEL Cool Agitator

Analysis

Agilent 7890 GC coupled to Agilent 5977 MSD with Extraction EI Source

SPME parameters

- Incubation time: 10 min
- Incubation Temperature: 10°C
- Agitation Speed: 500 rpm

GC/MS parameters

DB-5MS column 30 m x 0.25 mm x 0.25 µm
Oven 35°C ramped at 6°C/min to 260°C.
MS Scan acquisition (m/z 35-500)

MATERIALS

- GERSTEL SPME 20mL vials fitted with Extratech™ dedicated vacuum closures for SPME fibers as shown in Figure 3
- SPME Fibre: DVB/Carboxen/PDMS 50/30 µm; Stableflex
- Custom test mixture covering a range of analytes as shown in Table 1. Stock solution (100 µg/mL) prepared in ethanol.
- Test samples:

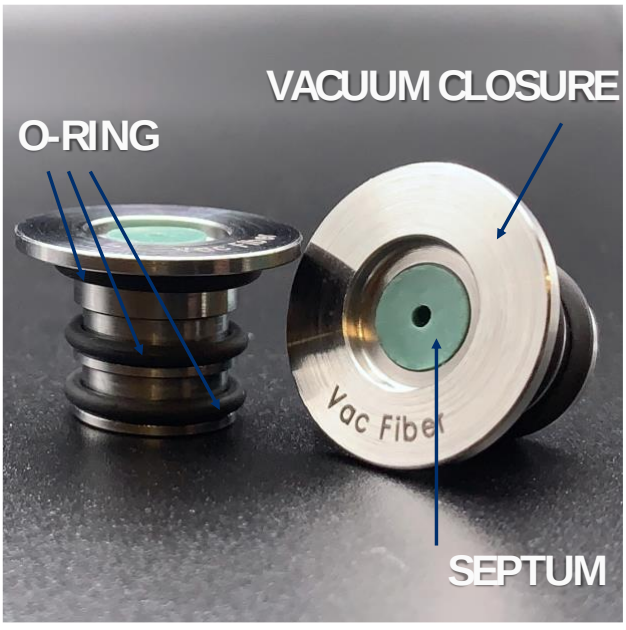


Figure 3: Extratech vacuum closures to work with Vac-HS-SPME

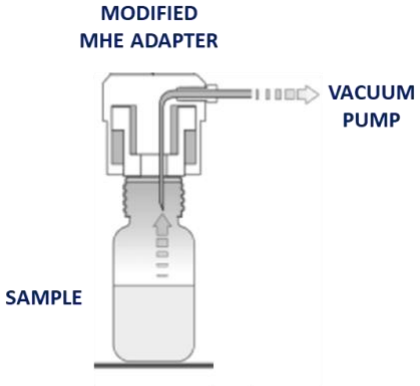
Table 1: Analytes included in custom test mixture to assess performances on Vac-HS-SPME

#	NAME	CAS NUMBER	MOLECULAR WEIGHT [amu]	BOILING POINT [°C]	K_H [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

RESULTS AND DISCUSSION

STAGE I: AUTOMATED Vac-HS-SPME DEVELOPMENT

Options were investigated for hardware and software to automate the evacuation step prior to HS-SPME-GC-MS analysis. A modified Multiple Headspace Extraction (MHE) Adapter in combination with an MHE holder and a remotely controlled vacuum pump was used to apply the vacuum to the vial headspace through the Fiber closure. The MHE adapter is a purge tool fitted with a side-holed needle connected to a gas line. The



* Modified Illustration 251 from CTC User Manual Edition 11

gas line was made gastight by the addition of a custom O-ring, and it was connected to a vacuum pump controlled remotely by the GERSTEL Maestro Software.

Figure 4: Modified MHE Adapter to apply vacuum to SPME vials.

To perform the evacuation step, a line of action commands were compiled in Maestro to move the vial to the dedicated evacuation station, apply the MHE adapter on the vial, activate the vacuum pump for the selected amont of time and at the selected pressure, and once completed, move the vial back to the tray to perform the standard SPME analysis as shown in Figure 5. All these commands were embedded in the GERSTEL parameters of the SPME-GC-MS method.

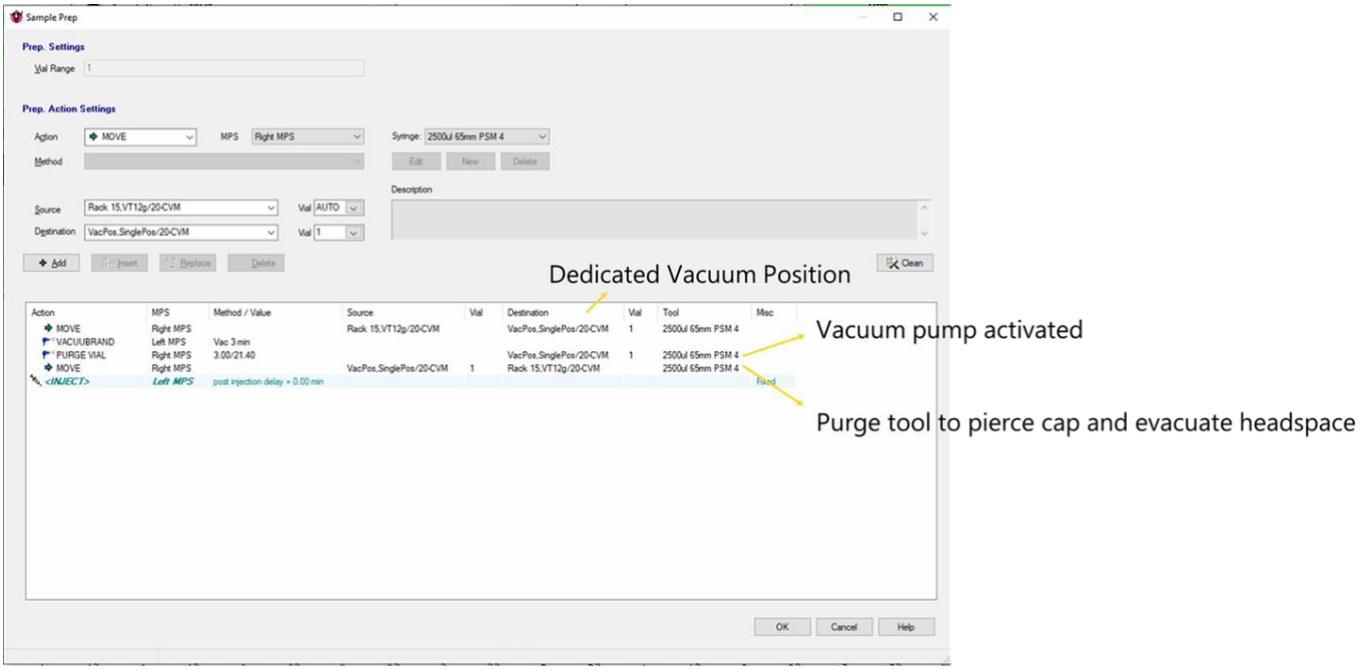


Figure 5: Sequence of action commands to perform the vial evacuation in GERSTEL Maestro software.

STAGE II: COMPARISON MANUAL vs AUTOMATED Vac-HS-SPME

Once the automated evacuation was fully developed, vacuum performances were compared between the manual and the automated approach. Two sets of five empty vials were capped with brand-new Fiber closures and the vacuum was applied, using the same pump settings, for both the manual and automated GERSTEL MPS approaches. Internal vacuum within the vials was measured using a digital manometer fitted with a needle to pierce the closure septum. Average vacuum for the manual approach was – 520 mbar ± 8 mbar and the average vacuum for the automated approach was -528 mbar ± 8 mbar, showing no statistically significant differences (t-test two tail P= 0.14).

STAGE III: EVALUATION OF TEST MIXTURE

To assess the performances of the automated Vac-HS-SPME a custom-made test mixture featuring a range of analytes with differing properties (as given in Table 1) was analysed using the same SPME-GC-MS method at atmospheric pressure (AP-HS-SPME) and under vacuum conditions (Vac-HS-SPME). A bulk test mixture solution was prepared, spiking 100 µL of the stock solution in 100 mL of deionised water reaching a final concentration of 0.1 µg/mL. 5 mL of solution were aliquoted in 20 mL SPME vials which were then fitted with the Fiber closures for both the atmospheric (n=4) and the vacuum (n=4) approaches. Peak areas for the 11 target analytes were recorded and compared between AP-HS-SPME and Vac-HS-SPME. Figure 6 shows an

example Chromatogram for the two approaches and the peak area increase factor calculated as the Vac-HS-SPME Peak Area dived by AP-HS-SPME Peak Area.

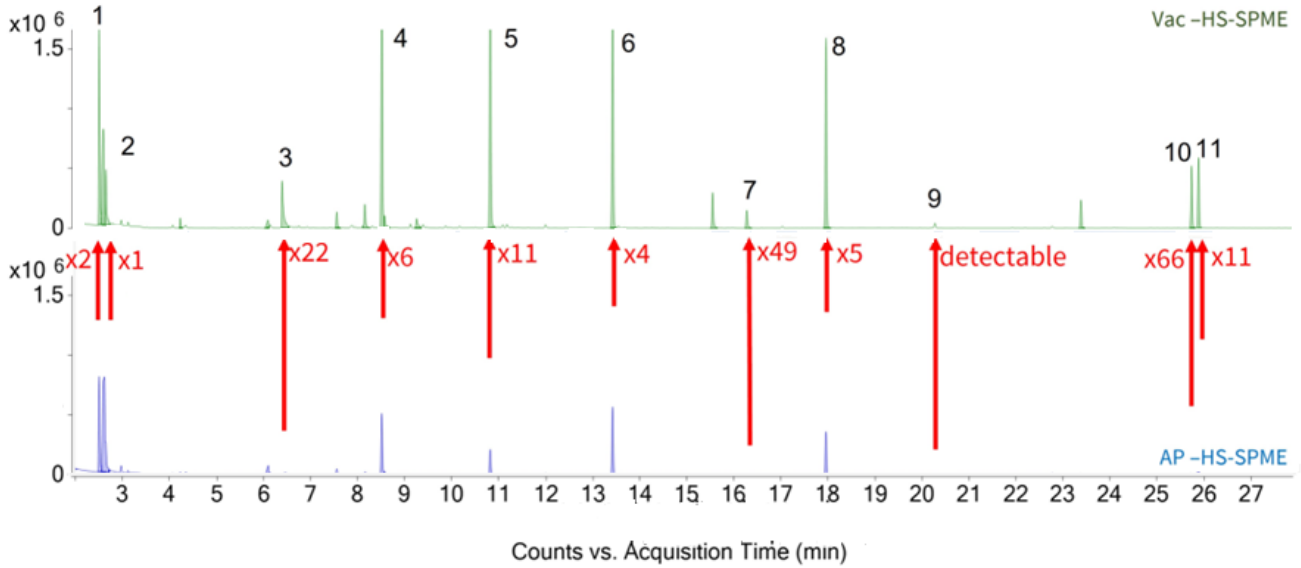
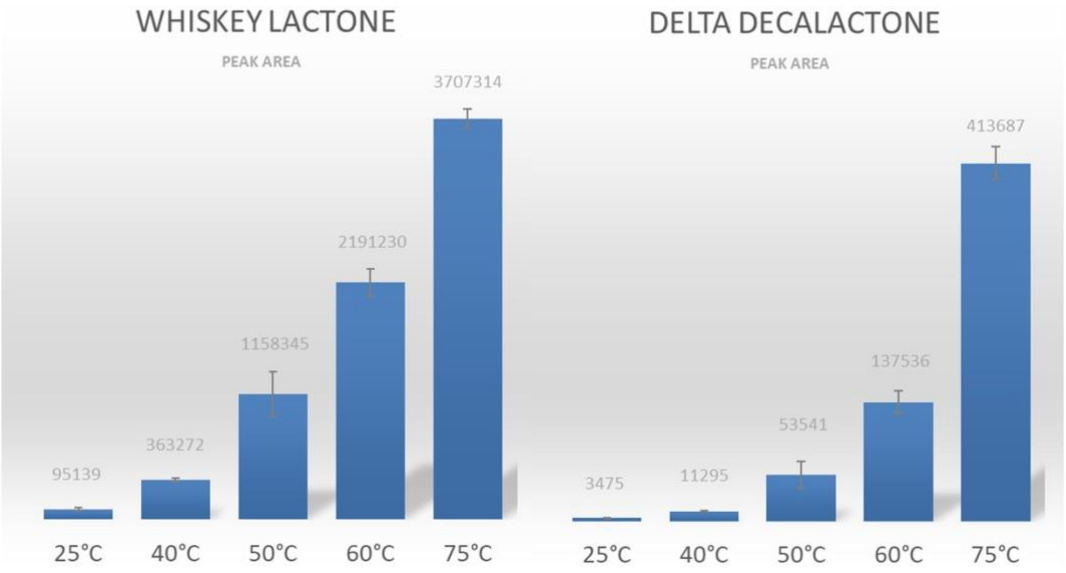


Figure 6: Chromatograms for AP-HS-SPME (bottom) and Vac-HS-SPME (top) for the investigated test mixture. The peak area increase factor for each of the targeted analytes as labelled in Table 1, are shown in red.

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.

An additional experiment was then performed to investigate whether increasing the SPME incubation temperature was enough to drive the lactones into the headspace and allow capture on the fiber without vacuum. Figure 7 summarises the peak area results for the Whiskey lactone and the Delta Deca lactone for



AP-HS-SPME at different incubation temperatures, ranging from 10°C up to 75 °C, with a 30 minute extraction time

Figure 7: Peak Area trends for Whiskey Lactone and Delta Decalactone with increased extraction temperature for AP-HS-SPME

Whilst it is clear higher temperatures are needed to transfer semivolatile analytes into the headspace, this approach is not ideal or in some cases even suitable. Particularly for food applications, an increase in temperature needs to be avoided to prevent the introduction of artefacts and shorter extraction times even at sub-ambient temperatures are required to provide an unaltered sample profile.

CONCLUSIONS

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.

Further, it has been demonstrated that the Vac-HS-SPME technique may give rise to significant analytes which are previously not seen with atmospheric pressure SPME approach.

The lure of this technique is enhanced by the possibility of automating the evacuation step and including it seamlessly within the standard SPME-GC-MS method.

This preliminary work allowed full automation of the evacuation step using the existing GERSTEL hardware and software and has been proven to be robust and reproducible. Furthermore, initial experiments on a custom-made test mixture have shown significant improvements in response for semi-volatile analytes, such as lactones.

The next stage of this work plans to specifically address the analysis of lactones in dairy products to showcase the added value offered by automated Vac-HS-SPME for a food application demanding the use of sub-ambient temperature for the sample extraction.

For further details or to arrange a proof-of-concept demonstration using your samples, please contact us at:

CONTACT DETAILS

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