Chromatography Technical Note No AS126

Automation of Headspace Solvent Micro-Extraction using Gerstel Multipurpose Sampler

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Introduction

The need for increased sensitivity and selectivity is a constant challenge facing many industries. One such technique that is becoming more popular amongst analytical scientists to overcome these challenges in a variety of sample types, particularly complex, dirty matrices is Single Drop Micro-Extraction (SDME).

SDME involves suspending a microdrop (typically 1-3µl) of solvent from the tip of a syringe in either a liquid sample, or the headspace above it. The intent is that analytes in the liquid sample or headspace migrate into the microdrop of solvent resulting in an enriched sample that can be retracted back into the syringe and injected directly onto a Gas Chromatography or Liquid Chromatography system for analysis.

This application note will focus on Headspace Solvent Micro-Extraction (HSME) for the analysis of volatiles in aqueous based sample systems. This technique can be described as occurring over 4 stages;

1. Sample Incubation - analytes are driven into the headspace
2. Enrichment - analytes are extracted into microdrop during the exposure period
3. Solvent Recovery - the microdrop containing the analytes is retracted back into syringe
4. Injection - Direct injection onto GC System.

Recovery of the drop back into the barrel of the syringe requires the needle tip to have a relatively large surface area. Hamilton 10ul syringes with a 26s gauge and a number 2 point style have been shown to be suitable for this purpose.

Choice of solvent is a critical aspect of the HSME procedure and subsequent analysis. Some important considerations when selecting the microdrop extraction solvent include:

- Solubility of target analytes in solvent to ensure analyte recovery
- Purity and boiling point of solvent to prevent target analyte interference during chromatographic analysis
- Boiling point of solvent to prevent evaporation during the extraction process
- Suitability for GC analysis

For these reasons, high molecular weight hydrocarbons such as 1-octanol and n-hexadecane have routinely been used to assess the capabilities of the technique.

Figure 1 shows a schematic diagram of how SDME works.

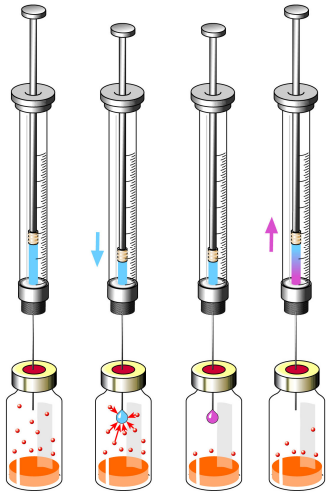

Figure 1 Operation of SDME

To date, most of the HSME work performed has been conducted using a manual procedure, which has the potential to introduce variation and prevents large numbers of samples being run efficiently. This application note explores the possibility of automating this technique using a Gerstel Multi Purpose Sampler (MPS) coupled to an Agilent GC-MS.

Gerstel Maestro Software Development

After an evaluation of the current Gerstel MPS Maestro software, it was apparent that the software was not able to perform the desired commands required for HSME. However, a version of the Maestro software was written by Gerstel to allow exposure (for a specified time) and aspiration of the microdrop prior to injection. As such, the instrumentation was now able to perform the basic command required for HSME.

Instrumentation

Agilent GC-MSD 5975C inert MSD with Triple-Axis Detector EI source (Agilent GC 7890A).
GERSTEL MPS 2 XL-*xt*
10ul GC Syringe type 2
Agilent Split/Splitless inlet
Maestro software (1.4.22.2) – Integrated.
Agilent MSD Chemstation E02.02 1431

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Figure 2: – Analytical solution for Headspace SDME analysis.

Methodology

A series of standards containing benzene, ethyl benzene, Toluene, o-xylene, m-xylene and p-xylene were prepared from a BTEX stock standard (2mg/ml in MeOH; Supelco 47993) at the following concentrations; 1.5ng/ml, 15ng/ml, 30ng/ml and 90ng/ml in water.

To assess the repeatability of the automated HSME procedure, six replicate samples at 15ng/ml were prepared by dispensing 15ml of the 15ng/ml BTEX standard solution into a 20ml headspace vial. The vials were sealed with a headspace vial cap and incubated at 50deg C for 10 minutes.

To gauge the linearity of the technique, samples at 1.5ng/ml, 15ng/ml, 30ng/ml and 90ng/ml were prepared in triplicate by dispensing 15ml of each of the four BTEX standard solutions into 20ml headspace vials. The vials were sealed with a headspace vial cap and incubated 50deg C for 10 minutes.

A 1ul drop of n-hexadecane was suspended in the headspace above each sample solution for 90 seconds and injected directly onto the GC-MS for analysis. The chromatographic conditions were as follows:

- 30m Wax Column 0.25mm x0.25um film thickness
- Carrier Gas - Helium
- Flow Rate – 1 ml/min
- Inlet Mode – Split (5:1)
- Total Runtime – 24 min
- MSD Mode – Selected Ion Monitoring (SIM)

Repeatability of Automated HSME (aHSME)

Table 1 below shows absolute peak area data for each of the target analytes within the samples.

Table 1: Repeatability of automated Headspace Solvent Micro-Extraction using a Gerstel MPS and GC-MS

	Analyte Absolute Peak Areas					
Replicate	Benzene	Toluene	o-xylene	m-xylene	p-xylene	Ethylbenzene
1	23814	101285	277241	226734	246372	193779
2	26091	111804	307903	253255	278139	218210
3	27680	117297	315722	260005	282361	221735
4	19903	86646	239172	200824	212183	169997
5	28145	121825	329168	270902	309134	235106
6	28253	121192	331482	272034	298367	233712
Mean	25648	110008	300115	247292	271093	212090
%RSD	13	12	12	11	13	12

The precision is acceptable, however given the variation associated with the extraction and GC injection process, use of an external standard is essential. To illustrate this, Benzene was assigned as an Internal Standard and the Peak Area Ratios for the 5 analytes were determined to correct for sample to sample variations (refer to Table 2).

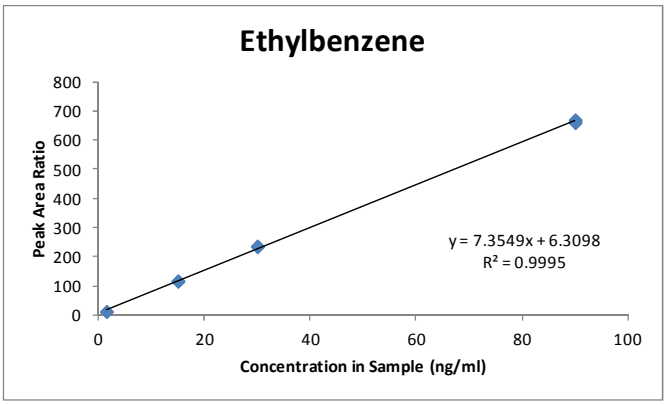
Table 2: Repeatability of automated Headspace Solvent Micro-Extraction using a Gerstel MPS and GC-MS (corrected using analyte Benzene as Internal Std)

Replicate	Toluene	o-xylene	m-xylene	p-xylene	ethylbenzene
1	4.25	11.64	9.52	10.35	8.14
2	4.29	11.80	9.71	10.66	8.36
3	4.24	11.41	9.39	10.20	8.01
4	4.35	12.02	10.09	10.66	8.54
5	4.33	11.70	9.63	10.98	8.35
6	4.29	11.73	9.63	10.56	8.27
Mean	4.3	11.7	9.7	10.6	8.3
%RSD	1.0	1.7	2.4	2.6	2.3

Precision is significantly improved using internal standard as expected.

Linearity of aHSME

Figure 3 shows the linearity of the HSME procedure for Ethylbenzene (Range 1.5 to 90 ng/ml BTEX)



An R2 value of 0.9995 demonstrates the potential of the technique with respect to its capability for quantification. Table 3 show the R2 values for each of the other analytes in the samples (internal standard corrected).

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Table 3: Regression coefficients for each analyte

Analyte	R2 Value
Toluene	1.000
o-xylene	0.999
m-xylene	0.999
p-xylene	0.999

Limit of Quantification of aHSME

The limit of quantification for the procedure was determined by analysing the 1.5ng/ml sample solutions as part of the linearity assessment and observing a s/n greater than 20 for each analyte of interest.

Conclusion

This initial data demonstrates that Headspace Solvent Micro-Extraction has the potential to be fully automated. A Design of Experiment is required to fully understand the quality critical parameters in order to understand the robustness of the method and potential application to the routine analysis of samples.

In addition to Headspace Solvent Micro-Extraction, – Immersive Solvent Micro Extraction could also be possible for trace semi volatile analysis.

We would like to thank Susanne Rose and Manfred Schwarzer at Gerstel for developing the new version of Maestro software to make automated HSME possible. We would also like to thank Alan Lockley at Anatune for modifying a 10µl GC syringe into a type 2 style needle.

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