APPNOTE

GERSTEL
MAKING LABS WORK

GERSTEL AppNote 102

Automated Solid Phase Microextraction using the GERSTEL MPS Prepstation and MAESTRO Software

Jaqueline A. Whitecavage and John R. Stuff

GERSTEL, Inc., 701 Digital Drive, Suite J, Linthicum, MD 21090, USA

Keywords

Automation, Solid Phase Microextraction, MAESTRO Software, GC/MS, Automated Sample Preparation

Abstract

Solid phase microextraction (SPME) is a powerful technique for the analysis of trace components in a wide variety of matrices. The types of compounds which can be analyzed by this technique ranges from non-polar to semi-polar materials due to the different fiber materials available. Most applications favor headspace over immersion sampling, therefore the analytes must be volatile enough to partition into the headspace of the vial being sampled. Often, heating and agitation of the sample are required. Quantification can be a challenge due to matrix effects. Automation of these and other steps can help with sample throughput, precision and accuracy.

The MPS PrepStation is a dual-rail/dual robot configuration of the GERSTEL MPS MultiPurpose Sampler. The PrepStation is fully programmable, combining automated sample preparation and sample introduction. One MPS rail of the MPS PrepStation is an automated liquid sample handler that performs a wide range of sample preparation functions. The second MPS rail can provide several automated sample introduction options, depending upon the selected MPS hardware configuration.

The new GERSTEL MAESTRO software provides users control software with a simplified user interface combined with significantly expanded capabilities. The sample preparation functions have been expanded, are easy to set up and have been extended to provide more operational flexibility.

This paper illustrates the use of automation to simplify sample preparation steps, such as derivatization, heating, agitation, and standard addition. Specific examples using solid phase microextraction are shown.

GERSTEL AppNote 102

Examples

Figure 1 shows the hardware configuration used for the examples presented in this paper. The left (lower) rail holds the SPME fiber and is used to inject the sample. The right (upper) rail can be configured with any size liquid syringe ranging from 1.2 to 1000 µL, and is used for sample or standard preparation.

Figure 1: Hardware configuration.

The MAESTRO software is integrated into the Agilent ChemStation where it can be accessed from two pull down menus. The GERSTEL and Agilent parameters are stored as part of a single method. A single sequence table controls all PrepBuilder and instrument parameters. The software gives the operator control of pertinent SPME parameters, along with the ability to sample directly from any sample vial in any tray on the autosampler. The software allows the user to bake out the fiber or select pre- or post-extraction derivatization with a simple check of a box.

Example 1: Trace Sulfur Compounds in IPA - Automated Sample Prep

In this example, trace sulfur compounds were causing an off odor when the product, 2-propanol was diluted to its final concentration of 10%. A 1000 µL liquid syringe was used to add 500 µL of sample or standard (thiophene) to 20 mL headspace vials, which were prefilled with 4.5 mL of water. After sample/standard addition, the vials were incubated for 10 minutes at 60 °C. The samples were extracted for 20 minutes with a 50/30 µm DVB/Carboxen/PDMS fiber. Figure 2 shows a resulting chromatogram in which six sulfur compounds were identified. The thiophene in the sample was quantitated at 45 ppb from a 5-point calibration curve (r^2 = 0.998). The RSD was 3.0% for n=3 samples.

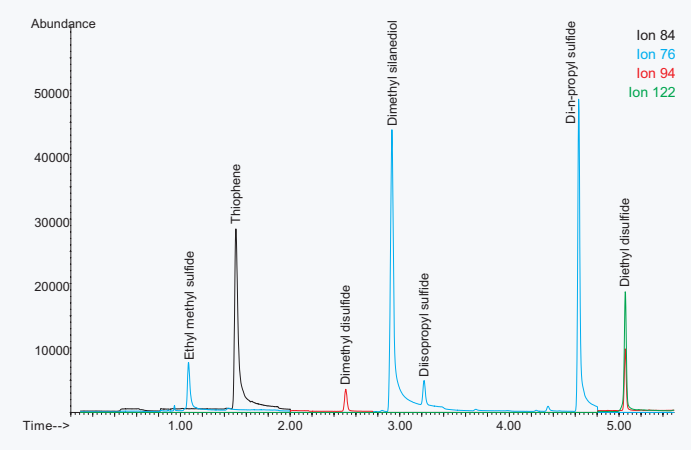

Figure 2: Chromatogram of sulfur compounds in IPA.

Example 2: Active Ingredients in Mouthwash - Automated Sample/Standard Prep.

In this example, the active ingredients in a mouthwash were analyzed for QC purposes. A calibration curve was built using a single standard. The right (upper) rail was fitted with a 10 µL liquid syringe that was used to add 5 uL of sample or varying amounts of standard to crimped, empty 20 mL headspace vials. After sample/standard addition, the vials were incubated for 20 minutes at 100 °C. The vials were extracted for 5 minutes with a 65 µm DVB/PDMS fiber. Figure 3 shows a resulting chromatogram. Figure 4 shows the calibration curve (r^2 = 0.993) resulting from the standards prepared by the PrepStation. The amount of methyl salicylate was quantitated in the sample with 1.8% RSD for n=5. For comparison, samples were prepared manually and the precision was 5.3%, n=5.

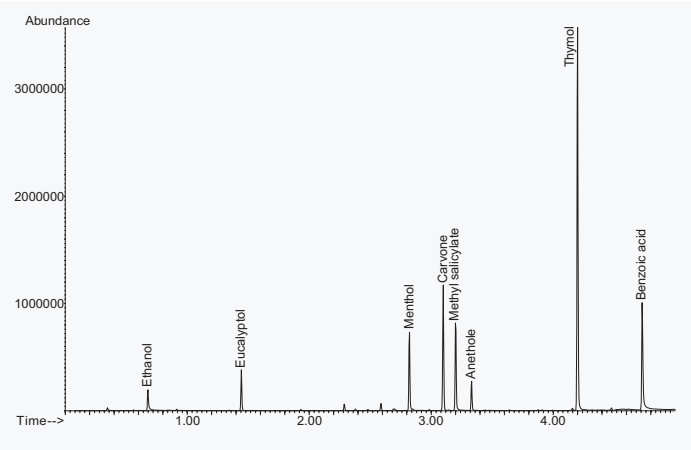

Figure 3: Chromatogram of active ingredients in mouthwash.

GERSTEL AppNote 102

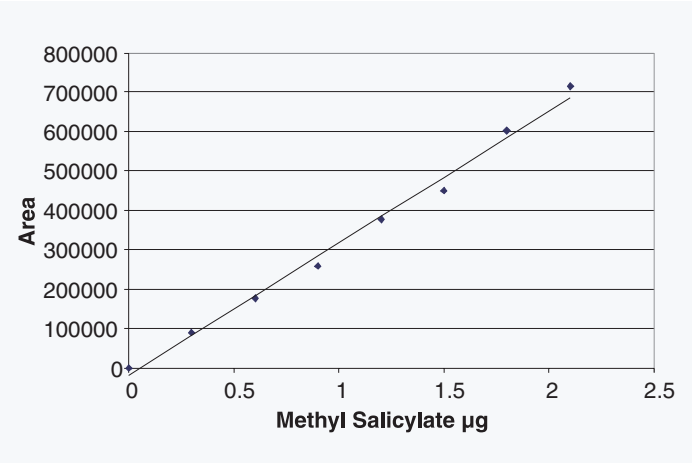

Figure 4: Calibration curve for methyl salicylate.

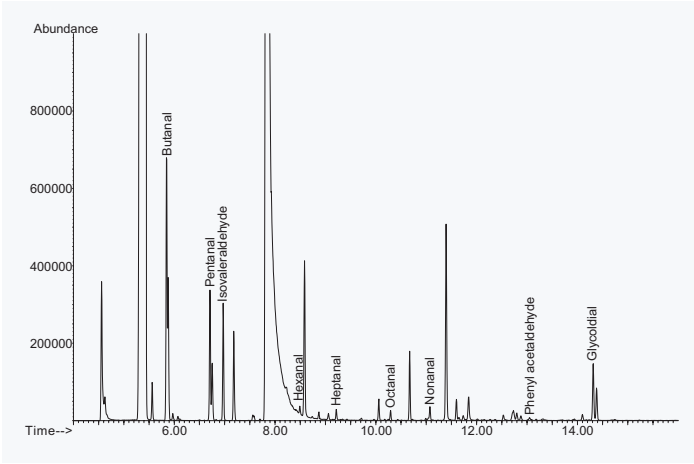

Figure 5: Chromatogram of derivatized wine sample.

Example 3: Aldehydes in Wine - Automated Sample/Standard Prep with Pre-Extraction Derivatization.

The perceived aroma and flavor of a wine is due in large part to the volatile organic compounds found therein. Aldehydes produced in the wine making process are an essential part of a wine's aroma profile. Aldehydes in alcoholic beverages can be detected using on-fiber derivatization with O-(2,3,4,5,6-pentafluorobenzyl) hydroxylamine (PFBOA) forming an oxime derivative. The pre-extraction derivatization is easily automated by selecting a checkbox in the Maestro software. The Prepstation was used to build a six point calibration curve for isovaleraldehyde, an aldehyde which imparts a „green" aroma to wine. This aldehyde was quantitated in several wines from Maryland wineries. The right (upper) rail was fitted with a 10 µL syringe. It was used to add varying amounts of standard to crimped 20 mL headspace vials filled with a 10% ethanol/water solution. Prior to extraction, a 65 µm DVB/PDMS fiber was loaded with derivatizing reagent by placing it in the headspace of a vial containing 60 mg/L PFBOA in water for 10 minutes at 50 °C. The samples were incubated for 10 min at 50 °C, then extracted for 5 minutes.

Figure 5 shows an example chromatogram. A six point calibration curve generated using the PrepStation was linear with an r^2 value of 0.993. Table 1 shows the amount of isovaleraldehyde found in three different wines, along with the % RSD for n=5 samples.

Table 1: % RSD of isovaleraldehyde in three different samples.

Sample	Isovaleraldehyde [ppb]	% RSD
Winery 1: Cabernet Sauvignon	5.8	3.5
Winery 2: Cabernet Sauvignon	5.1	2.8
Winery 3: Cabernet Franc	7.4	2.7

Example 4: Drugs of Abuse in Saliva - Automated Addition of an Internal Standard, Salt Water, Base and Derivatizing Reagent. Preparation of Standard Curve.

SPME can be used as an effective technique for the trace detection of drugs of abuse in saliva samples. The effectiveness of the SPME extraction can be enhanced by the addition of salt and base. An internal standard can be added for quantitation, and a derivatizing reagent added for those analytes which are too polar or too involatile to partition into the sample headspace. All these steps can be automated using the GERSTEL PrepStation. For this example, 300 uL of saliva were added to a 10 mL headspace vial. The right (upper) rail was fitted with a 100 µL liquid syringe. We used a two step sampling approach, where the first SPME extraction is used to extract analytes that do not require derivatization, and the second step with addition of a derivatizing agent targets amphetamine derivatives. An example PrepSequence Action List is shown in Figure 6.

GERSTEL AppNote 102

PrepSequence Action List

ACTION	METHOD	SOURCE	DESTINATION
PREP Vials 1 - 1			
(R) ADD	Add Salt Water	1 @ Wash2	1 @ Tray1,VT32-10
(R) ADD	Add Base	1 @ SolvRes1	1 @ Tray1,VT32-10
(R) ADD	Add Internal Std	1 @ SolvRes2	1 @ Tray1,VT32-10
(L) MIX	Mix Sample Left		
(L) INJECT	Drugs_SPME_081506_Scan.m\maestrc	Tray1,VT32-10	Rear
END			
PREP Vials 2 - 2			
(R) ADD	Add Salt Water	1 @ Wash2	2 @ Tray1,VT32-10
(R) ADD	Add Base	1 @ SolvRes1	2 @ Tray1,VT32-10
(R) ADD	Add Internal Std	1 @ SolvRes2	2 @ Tray1,VT32-10
(R) ADD	Add 55 uL Std	1 @ Standard	2 @ Tray1,VT32-10
(L) MIX	Mix Sample Left		
(L) INJECT	Drugs_SPME_081506_Scan.m\maestrc	Tray1,VT32-10	Rear
END			
PREP Vials 1 - 2			
(R) ADD	Add Derivative	SolvRes3	Tray1,VT32-10
(L) MIX	Mix Sample Left		
(L) INJECT	Drugs_SPME_081506_Scan.m\maestrc	Tray1,VT32-10	Rear
END			

Figure 6: Example prep sequence for saliva samples.

Vial 1 represents the preparation of a sample, where 100 µL of saturated salt water, 20 µL of 0.5 N KOH, and 20 µL of internal standard are added to the vial, the vial contents are mixed and subsequently extracted with a SPME fiber and analyzed. Vial 2 represents preparation of a standard for the calibration curve where an „Add Standard" step has been inserted prior to mixing. The second part of the analysis is conducted by adding 20 µL of butyl chlorformate (diluted 1:10 in acetone) to the sample, as a derivatizing reagent. The samples were incubated at 60 °C for ten minutes and extracted for 20 minutes using a 100 µm PDMS fiber. Figure 7 is a typical chromatogram for the first extraction showing PCP, methadone, amitriptyline, and imipramine.

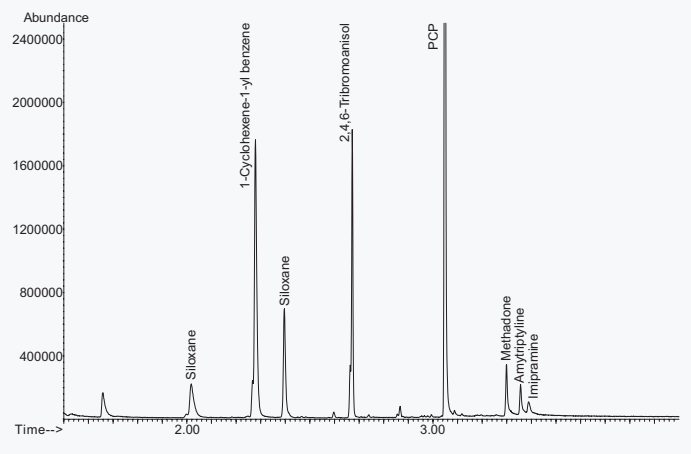

Figure 7: Chromatogram for 1st extraction of saliva.

GERSTEL AppNote 102

Figure 8 shows a typical chromatogram for the second extraction including the butyl chloroformate derivatives of amphetamine and methamphetamine. Table 2 shows quantitative results for this example.

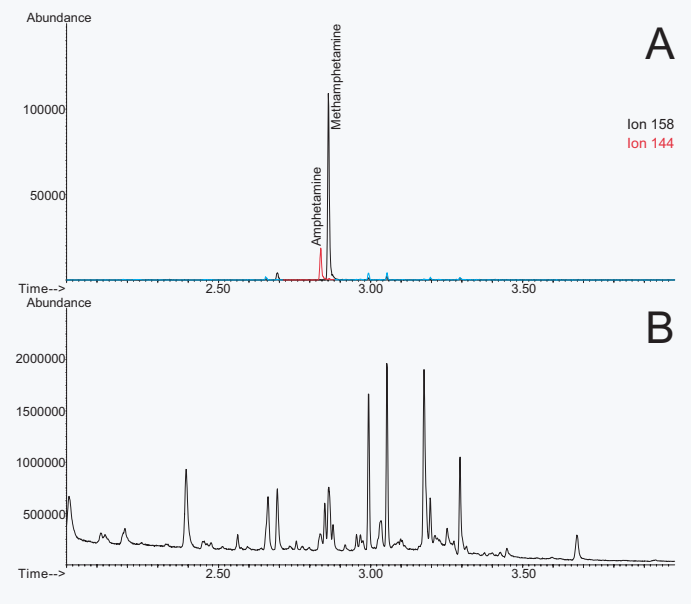

Figure 8: Chromatogram for 2nd extraction of saliva with derivatization, EIC (A) and TIC(B).

Table 2: Quantitative results.

Analyte	r^2 (5pt curve)	Found [µg/mL]	Actual [µg/mL]
PCP	0.996	0.91	0.98
Methadone	0.993	1.01	0.98
Amitriptyline	0.992	0.97	0.98
Imipramine	0.986	0.98	0.98
Amphetamine	0.995	1.07	0.98
Methamphetamine	0.987	1.04	0.98

Conclusions

The GERSTEL MPS PrepStation and MAESTRO software provide:

- A unique combination of simplified programming and advanced automation capabilities for SPME
- Software control fully integrated into Agilent Technologies ChemStation
- All autosampler parameters are saved as part of the ChemStation method
- A single sequence table ties together all sample, method and data file information

Automated Sample Prep Functions provided by the MAESTRO software and MPS autosampler:

- Dilution
- Derivatization
- Agitation
- Rinsing
- Liquid-Liquid Extraction
- Heating
- Spiking
- Standard Addition
- Standard preparation

References

[1] P.A. Martos and J. Pawliszyn, Anal. Chem. **1998**, 70, 2311

[2] P. Vesely, L. Lusk, G. Basarova, J. Seabrooks, and D. Ryder, J.Agric.Food.Chem., 51, 6941-6944 (**2003**)

[3] N. Fucci, N. de Giovanni, and M. Chiarotti, Foren.Sci.Int., 134, 40-45 (**2003**)

[4] M. Yonamine, N. Tawil, R. Lucia de Moraes Moreau, and O. Alves Silva, J.Chrom. B, 789, 73-78 (**2003**).