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Evaporative Concentration of Substances Listed in the European Water Framework Directive (2000/60/EC and 2008/105/EC). A Performance Comparison Between an Automated System and a Manual System.

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KEYWORDS
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ABSTRACT
Having to reach ever lower limits of detection is a daily challenge in modern laboratories. In order to succeed in obtaining sufficiently sensitive analysis methods, sample preparation techniques such as Solid Phase Extraction (SPE) or Liquid-Liquid Extraction are often used as concentration steps. The concentration factor achieved in these cases depends on the amount of solvent used for analyte elution from the SPE cartridge or for liquid extraction. Following the extraction step, further concentration of analytes can be achieved by reducing the amount of solvent left in the extract. This is typically achieved by evaporation. Such a concentration step can contribute significantly to improved limits of detection for the overall analytical method.

For the evaporative concentration step, commercially available rotary evaporators as well as custom solutions are widely used. These are mainly stand-alone systems for manual operation. The GERSTEL MultiPosition Evaporation Station (mVAP) in combination with the GERSTEL MultiPurpose Sampler (MPS) now offers fully automated concentration of sample extracts. The system enables complete automation

of all sample preparation steps including introduction to an a LC- or GC-system. The evaporation is controlled by controlling the applied vacuum leading to reproducible results independent of the solvent used. The user can also benefit from a real increase in laboratory efficiency, since batches of samples can be processed automatically overnight. In this Application Note we demonstrate the performance of the mVAP and compare the results with those obtained using a commercially available evaporation system based on nitrogen flow.

INTRODUCTION

Sample preparation using appropriate processing steps generally achieves many goals: Sample compounds of interest are isolated, extracted, and concentrated before being transferred to the analysis system in a form and manner, in which they can be determined at the required limits of detection. One useful way to concentrate analytes is reduction of the sample- or extract volume through solvent evaporation. In addition to the concentration effect, solvent evaporation enables a solvent exchange in which the residue is taken up in a small volume of a different solvent. The used solvent should be compatible with the analytical sample introduction, with the separation technique, and with the detection system used in order to achieve the best possible results.

A range of techniques are available to remove excess solvent. One of the most widely used is rotary evaporation. The functional principle is solvent evaporation under reduced pressure in a rotating flask, which is partially submerged in a temperature controlled water- or oil bath. The flask is rotating at an angle of approximately 45 degrees, the rotation thus provides a higher solvent surface area and an even temperature distribution inside the flask leading to a higher rate of evaporation and helping to avoid spattering in the boiling process.

Another widely used technique is the concentration of samples in an open test tube or vial, through which an inert gas, usually nitrogen, is blown above the sample surface. Evaporation temperature control is achieved using a heating block or a water bath.

Generally attention to detail is required when concentrating extracts, because analytes may be lost through thermal degradation or evaporation. The key parameters influencing the process are the duration of the evaporation process, the temperature, the reduced pressure or the flow of nitrogen, respectively, as well as the final evaporation volume [1]. For example, if a too high evaporation temperature is chosen, substances with low boiling point could be lost [1,2].

Standard technical solutions are commercially available for the procedures explained above, but most of these require manual steps. GERSTEL has recently developed a multi-position evaporation station (mVAP) for the GERSTEL MultiPurpose Sampler (MPS). The MPS automates evaporative concentration of samples and extracts in combination with additional sample preparation steps and sample introduction to the GC/MS or LC/MS analysis system [3].

Instrumentation

mVAP: GERSTEL Multi-Position Evaporation Station. Using the mVAP in combination with the GERSTEL MPS enables automated processing of batches of samples placed in commercially available 2 mL, 4 mL and 10 mL vials. Method parameters for sample preparation steps, such as addition of liquid standards or derivatization reagents, SPE or DPX (Disposable Pipette Extraction), evaporation, and sample introduction are set up by mouse-click using GERSTEL MAESTRO Software.

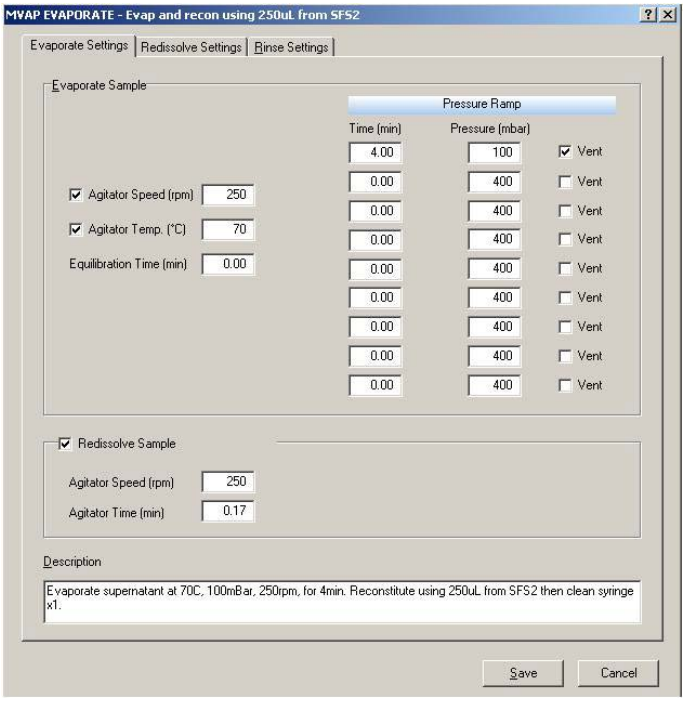

Figure 1. MAESTRO mVAP method parameter window.

The controlled, user defined vacuum can be set to values as low as 50 mbar. This, in combination with user defined temperature, agitation speed, and evaporation time, enables the user to achieve efficient evaporation and reproducible evaporation rates from sample to sample.

mVAP is used in combination with the PC 3001 Vario vacuum pump (Vacuubrand GmbH & Co. KG, Germany) under integrated GERSTEL MAESTRO control. All commonly used solvents such as acetone, methanol, and n-hexane, among many others, can be evaporated using mVAP. The efficient orbital shaking of samples in the device ensures homogeneous temperature distribution within the sample, preventing overheating and uncontrolled boiling, while increasing the evaporation surface. After the evaporation step, solvent exchange can be performed automatically, including rinsing the walls of the sample vial. Rinsing of the vial walls is recommended even if no solvent exchange has been performed. Finally, the concentrated samples are transported to a destination tray for further processing or for injection into an LC- or GC system.

Figure 2. GERSTEL Multi-Position Evaporation Station (mVAP).

Performance comparison using a reference system. For the performance comparison between mVAP and a commonly used evaporation device for laboratory use, 54 organic xenobiotics from different chemical classes were chosen. The choice was orientated along the line of substances listed in the European Water Framework Directive (2000/60/EC and 2008/105/EC) and included substances such as polycyclic aromatic hydrocarbons (PAHs), poly-brominated diphenyl ethers (PBDEs), halogenated hydrocarbons, some pesticides, as well as polychlorinated biphenyls (PCBs) [4,5]. An 8 mL aliquot of an acetone solution containing 5 µg/L of each compound was concentrated at 35°C.

For comparison, the evaporative concentration by the mVAP station (System 1) was performed at an absolute pressure of 200 mbar and orbital agitation speed of 250 rpm. After the evaporation has been completed, the glass wall of each sample vial was rinsed with solvent at 750 rpm for 1 minute. The reference device based on evaporation at a controlled temperature under a flow of nitrogen (System 2) was operated with a front pressure of 7 bar and a resulting flow of 20 mL/min.

Chromatography system/detector.
GC 6890/5973 MSD (Agilent Technologies)

Analysis conditions.

PTV	80°C, 12°C/s, 300°C (10 min) splitless glass liner, deactivated
Oven	50°C, 10°C/min, 300°C (10 min)
Column	MN Optima®-5-ms (Macherey-Nagel)

Compounds were identified based on their retention time, and up to four specific m/z values (SIM-Mode), of which one was used for quantification.

Results and Discussion

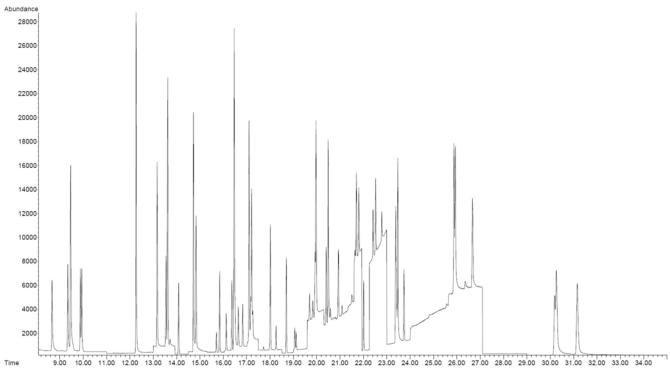

Figure 2. SIM Chromatogram of 54 target compounds, six internal standards, and a volumetric calibration (isotope dilution) standard.

Table 1 presents the recoveries and the relative standard deviations (RSDs) for both investigated systems. These results have been adjusted for volume deviations by a volumetric standard (fluoranthene-D_{10}). It can be seen that the two systems deliver comparable results for each sample, even for the most volatile substances, such as trichlorobenzenes, naphthalene, and hexachlorobutadiene. RSDs were consistently below 10 % for both systems. Even the time required for evaporation was comparable: 20 minutes (mVAP) and 22 minutes (N_2-based system).

Table 1. Analyte recovery in % after evaporation at 35°C of an analyte solution containing 5 µg/L of each compound in acetone (n = 4) .

Analyte	mVap (System 1)		Reference device (System 2)	
	Recovery	RSD	Recovery	RSD
1,3,5-Trichlorobenzene	100 %	3 %	101 %	3 %
1,2,4-Trichlorobenzene	100 %	4 %	100 %	3 %
Naphthalene	99 %	5 %	101 %	3 %
Hexachlorobutadiene	100 %	4 %	100 %	3 %
1,2,3-Trichlorobenzene	103 %	4 %	104 %	2 %
3,4-Dichloronitrobenzene	100 %	2 %	94 %	4 %
Acenaphthylene	97 %	3 %	97 %	3 %
Acenaphtene-D_{10}	100 %	2 %	98 %	3 %
Acenaphthene	100 %	3 %	99 %	3 %
Pentachlorobenzene	103 %	3 %	102 %	4 %
Fluorene	100 %	3 %	101 %	3 %
Trifluralin	105 %	2 %	105 %	2 %
4,4'-Dibromooctafluorobiphenyl	89 %	6 %	98 %	3 %
alpha-HCH	95 %	1 %	100 %	3 %
Hexachlorobenzene	100 %	1 %	101 %	3 %
Simazine	103 %	5 %	106 %	2 %
Atrazine-D_5	109 %	8 %	108 %	3 %
Atrazine	108 %	6 %	103 %	3 %
beta-HCH	102 %	3 %	104 %	3 %
gamma-HCH	102 %	4 %	107 %	5 %
Phenanthrene	102 %	1 %	102 %	3 %
Anthracene-D_{10}	97 %	3 %	99 %	3 %
Anthracene	97 %	3 %	97 %	4 %
delta-HCH	84 %	8 %	109 %	5 %
PCB 28	104 %	1 %	104 %	2 %
Alachlor	102 %	2 %	105 %	5 %
PCB 52	104 %	1 %	106 %	2 %
Chlorpyrifos-ethyl	109 %	3 %	103 %	5 %
Aldrin	100 %	2 %	100 %	1 %
Isodrin	95 %	1%	99 %	6 %
Chlorfenvinphos	101 %	3 %	96 %	7 %
Fluoranthene	103 %	2 %	104 %	1 %
PCB 101	104 %	1 %	108 %	3 %
Pyrene	104 %	1 %	105 %	3 %
alpha-Endosulfan	102 %	5 %	106 %	5 %
p,p'-DDE	104 %	2 %	106 %	3 %
Dieldrin	95 %	2 %	101 %	5 %
Endrin	96 %	3 %	101 %	6 %
beta-Endosulfan	98 %	5 %	98 %	3 %
p,p'-TDE	105 %	4 %	106 %	5 %
o,p'-DDT	102 %	3 %	104 %	3 %

Table 1 (cont.). Analyte recovery in % after evaporation at 35°C of an analyte solution containing 5 µg/L of each compound in acetone (n = 4) .

Analyte	mVap (System 1)		Reference device (System 2)	
	Recovery	RSD	Recovery	RSD
PCB 153	103 %	2 %	108 %	3 %
p,p'-DDT	105 %	3 %	109 %	5 %
PCB 138	100 %	3 %	108 %	5 %
Benzo[a]anthracene	106 %	4 %	106 %	4 %
Chrysene-D_{12}	105 %	2 %	109 %	3 %
Chrysene	105 %	5 %	106 %	4 %
PCB 180	97 %	3 %	108 %	3 %
Benzo[b]fluoranthene	103 %	4 %	104 %	6 %
Benzo[k]fluoranthene	97 %	5 %	103 %	4 %
Benzo[a]pyrene	108 %	4 %	105 %	3 %
Indeno[1,2,3-c,d]pyrene	98 %	5 %	102 %	6 %
Dibenzo[a,h]anthracene	102 %	5 %	103 %	5 %
Benzo[g,h,i]perylene	107 %	3%	108 %	5 %

An important difference between the two evaporation systems is the automation or lack thereof: The mVAP offers fully automated operation using an industry standard autosampler, while the conventional system based on evaporation under a flow of nitrogen requires manual loading and unloading of samples, offering no further automated sample preparation steps. However, the conventional system is able to process 50 samples at a time in one batch, which can offer an advantage if automation is not of critical importance. The mVAP module processes up to 98 samples automatically in batches of six. An overview and comparison of the systems is shown in table 2.

Table 2. Comparison between mVap and the reference N_2-system: System properties and required handling steps.

	mVap (System 1)	Reference device (System 2)
Evaporation under	Vacuum	Nitrogen Flow
Sample loading and unloading	Automated	Manual
Rinsing of glass walls	Automated	Automated/ Manual
Solvent Exchange	Automated	Manual
End of Evaporation	Time dependent	Time dependent
Max. # of Samples	98 (in batches of 6, overnight)	50 (one batch)
Temperature control of sample	Yes	Yes
Sample Vial Sizes	2 mL, 4 mL & 10 mL	10 mL (depending on inserts used)

Conclusion

In this work, the GERSTEL MultiPosition Evaporation Station (mVAP) was shown to offer performance fully comparable with a widely used commercially available evaporation station that is based on manual operation and evaporation under a flow of nitrogen. Among the advantages of the mVAP are: Complete integration into an automated sample preparation system; full automation of all steps including introduction to the GC/MS or LC/MS analysis system; and the capability to process up to 98 samples in unattended operation. All necessary steps for analyzing samples starting with extraction and evaporation and ending with injection into a chromatographic system can be performed automatically for improved laboratory efficiency and throughput.

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[5] Directive 2008/105/EC of the European Parliament and of the Council of 16 December 2008 on environmental quality standards in the field of water policy, amending and subsequently repealing Council Directives 82/176/EEC, 83/513/EEC, 84/156/EEC, 84/491/EEC and 86/280/EWG and amending Directive 2000/60/EC, of the European Parliament and of the Council

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