MULTIPLE SAMPLE PREPARATION STEPS MADE EASY BY AUTOMATION
or: From Crude Sample to (GC-MS) Analysis in „one" Work-Step

M.A. ALTHOFF[*, #], A. Bertsch[*] and M. Metzulat[*]

[*]Science Division, CBRN Defence, Safety and Environmental Protection School, Sonthofen, Germany
[#]Department of Chemistry, Ludwig-Maximilian University, Munich, Germany

ABSTRACT

In our study, we have set up a TriPlusRSH autosampler mounted on a GC-MS system using the new Method Composer Software and advanced scripting to automate all necessary sample preparation steps and subsequent injection of samples in the field of chemical disarmament.

Those include but are not limited to: liquid-liquid extraction (LLE), drying steps, solvent exchange and μ-SPE. Efficiency and accuracy of the methods were carefully evaluated and proven to outperform the respective manual steps by showing significant smaller standard deviations and variance levels. Tedious and error prone off line steps are eliminated. The setup engaged features brand new prototype equipment, e.g. a centrifuge to assist in phase separation for LLE.

Besides this, the automated workflow can easily be adapted to other sample preparation protocols for e.g. determination of octanol-water partition coefficients and be used amongst different instruments and chromatography data handling systems.

INTRODUCTION

Preparation and processing of samples prior to analysis is one of the most crucial steps in analytical chemistry and at the same time the most time consuming task (1); if it has to be done manually, it is often also one of the most strenuous steps. However, autosamplers are frequently applied only for subjecting the samples to the instruments instead of being used for sample preparation. New developments in miniaturization and automation are less frequently observed in routine applications, as those experiments are fixed by certified SOPs. Recently, μ-SPE cartridges were introduced (3) as well as a centrifuge (4) which are both compatible with existing autosamplers. Those can easily be integrated and operated via existing chromatography data systems or easy to use PAL software applications.

Especially, when investigating highly toxic substances, automation can help to **minimize health risks** for lab personnel.

Figure 1. Sample preparation work steps for aqueous samples according to the "blue book".(2)

PROTOTYPE EQUIPMENT

PAL **Centrifuge** operated at RCF = 5000 G (max.) was used for phase separation in LLE and liquid extraction of soil samples. ITSP® **μ-SPE cartridges** (C18-EC and Silica) were used in GC-MS(SIM) analysis of chemical warfare agents and degradation products thereof.

Figure 2. Centrifuge rotor for processing of 8 x 2 ml vials (left) and PAL icons of Method Composer, Script Editor and Script Executor software (right).

- Plunger drives solvents and sample through the cartridge at precise flow rates.
- Lower needle guide ensures accessing the same hole of the septum several times.
- Crimped-on 8 mm septum ensures a closed flow path to the SPE sorbent and facilitates cartridge transport.
- Needle guide eliminates headspace above the SPE sorbent (22 gauge ID).
- Sorbent is sandwiched between frits and filter membrane.
- The SPE column is just micro-volume.

Figure 3. Description of the μ-SPE principle according to ITSP® Solutions, Inc.(3)

MATERIALS AND METHODS

Experiments were performed on a Thermo Fisher Scientific GC-MS/MS (Trace 1310 with TSQ Duo) instrument equipped with a TriPlusRSH autosampler (Fig. 9 and 10). Analytes were VX, VG (Amiton) and HD (Mustard) and VX-Disulfide.

1) Samples were spiked with 10 ppm of the respective analyte.
2) μ-SPE experiments were done to i) remove hydrocarbon background and ii) to transfer analytes from water to organic phase.
3) Liquid-Liquid Extraction (LLE) experiments were performed to prove the effectiveness of the automated setup and phase transfer of non-polar analytes, e.g. mustard.
4) Liquid-Solid Extraction experiments were performed similarly to LLE experiments with the purpose to extract chemical warfare agents form soil or wipe samples.
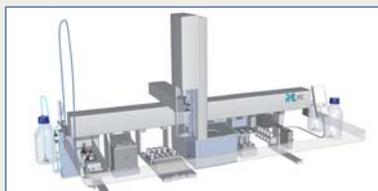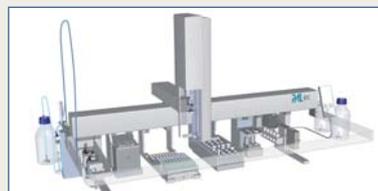
μ-SPE RESULTS

μ-SPE is not different from regular SPE! It's just about size. μ-SPE cartridges have a sorbent volume of 10-45 mg. Flow rates are precisely controlled by the autosampler plunger motor (as low as 1 μl/s). For example: 10 ml of raw sample can be enriched by a factor of 200 by final elution with only 50 μl. Moreover, this tiny volume can directly be derivatized without solvent evaporation.

Figure 4. Results of the μ-SPE method to remove hydrocarbon background (diesel fuel 10 mg/ml) of spiked (10 μg/ml) samples of VX and VG (Amiton) by silica cartridge.

Experimental workflows follow recommendation of the "blue book" with optimized conditions for the μ-SPE cartridges.

As a result μ-SPE offers higher recovery rates than standard SPE workflows compared to literature resources, faster processing times and lower solvent consumption.

Additionally, μ-SPE is also a superior technique with respect to the experimental effort.

HARDWARE LIMITATIONS:
- Filtration
- Controlled Evaporation

Table 1. Comparison of μ-SPE and standard SPE.

Parameter	μ-SPE	SPE
solvent volume	125 μl	3 ml
processing time	10 min	15 min
flow rate	1-100 μl/s	ca. 1 drop/s

Table 2. Recovery rates of chemical warfare agents. Literature values according to ref. (6).

Matrix (cartridge)	Recovery [% ± RSD]		
	VX	VG Amiton	HD Mustard
water (C18-EC)	56.5±0.4 24±10 (Lit.)	65.8±0.1 -	64.8±1.9 32±7 (Lit.)
diesel fuel (silica)	76.3±1.3	98.2±1.4	work-up impossible
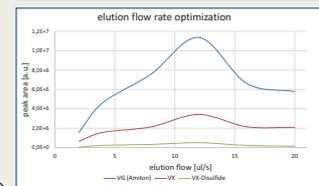
Chart 5. Elution flow rate optimization during μ-SPE method development for C18-EC cartridge (aqueous → organic).

LIQUID-LIQUID-EXTRACTION RESULTS

Figure 6. Exemplary workflow of the LLE experiment for aqueous samples.

- Add buffer and extraction solvent
- Vortex sample
- Centrifuge sample
- Take aliquot (upper or lower phase)
- Add to drying agent (sodium sulfate)
- Vortex in order to dry sample
- Take aliquot, add derivatization agent
- Agitate and heat sample
- Injection

Figure 7. Vials after i) extraction (left, billimex®), ii) centrifugation (middle) and iii) drying (vortexed) with Na_2SO_4 (right).

The developed LLE workflow can be universally applied to the extraction of analytes from aqueous phases to organic phases and visa-versa. Additionally, it can also be used for the effective extraction of solid materials, e.g.: soil or wipe samples.

Method transition to other LLE-procedures, e.g. octanol-water partition coefficients, can easily be performed by setting unnecessary workflow items optional.

Taking precise amounts of the lower or upper extraction phase can be achieved with billimex® LD/HD-AS vials (5).

HARDWARE LIMITATIONS:
- Filtration
- Controlled Evaporation
- G-force limit: 2000 G (10 and 20 ml Vials)

Table 3. Results of nerve agents VX and VG and blister agent Mustard (HD) extraction.

Matrix		Recovery [% ± RSD]		
		VX	VG	HD
tap water	pH 7	64.1±9.6	79.8±8.3	47.3±3.8
	pH 11	88.0±3.4	85.9±5.1	22.1±2.7
ground water	pH 7	96.0±3.4	99.9±3.6	51.5±4.0
	pH 11	98.7±1.4	99.9±1.8	50.7±1.4
soil	DCM	66.2±2.7	88.2±4.0	46.8±4.4
wipe	DCM	65.6±3.1	58.4±2.3	51.2±1.4
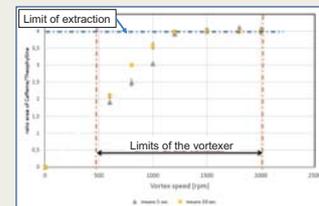
Figure 8. Results of the automated liquid-liquid extraction methodology. Noteworthy are the short extraction times of 5 to 10 sec. and completeness of extraction at 1200 rpm.

AUTOSAMPLER INSTRUMENT SETUP

Figure 9. Autosampler setup for LLE procedures: ATC, Agitator, sample rack, Centrifuge, Vortexer, Standard Wash Station and Fast Wash Station (left to right).

Figure 10. Autosampler setup for μ-SPE procedures: ATC, Agitator, μ-SPE rack, Tray-Cooler, Vortexer, Standard Wash Station and Fast Wash Station (left to right).

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