Automated Disposable Pipette Extraction (DPX)

Improved laboratory productivity

Disposable Pipette Extraction (DPX) is a fast and efficient SPE technique used for a wide range of applications such as drugs of abuse and therapeutic drug monitoring. Only 200 - 250 µL of sample is needed to reach the required limits of detection using a fully automated process. The extraction step is performed in 30 -60 seconds and the complete process including elution and rinse steps takes 3 - 6 minutes depending on the application. Additionally, automated DPX is performed during the GC or LC run of the preceding sample ensuring maximum throughput and best possible GC/MS or LC/MS system utilization. Elution requires only a small amount of solvent, which means that DPX effectively provides a concentration step. For many applications, such as pesticides in fruit and vegetables, solvent evaporation is not required. For more information about DPX system, please visit www.gerstel.com (Applications/ Technology/Extraction Techniques):

AppNote-2009-06
Comprehensive Analysis of Drugs of Abuse in Blood and Urine with Automated Disposable Pipette Extraction and HPLC/MS/ MS

AppNote-2009-01
Automated Multi-Residue Pesticide Analysis in Fruits and Vegetables by Disposable Pipette Extraction (DPX) and Gas Chromatography/Mass Spectrometry

AppNote-2008-11
Analysis of Drugs and Metabolites in Blood and Urine using Disposable Pipette Extraction

Visit GERSTEL at booth # 13/14

Intelligent Automated Sample Preparation

Fast, accurate, and highly sensitive LC/MS determination of PFCs

The determination of Perfluorinated Compounds (PFCs) in water according to the recognized international standard method (ISO/DIS 25101) is performed based on solid phase Extraction combined with HPLC-MS/MS determination. Application chemists have recently shown that if sample preparation and PFC determination is fully automated; the analysis can be performed more productively – and with significantly improved performance.

PFCs are creations of organic synthesis, developed in the R&D laboratory, produced in significant amounts and ubiquitous in the environment. PFC surfactants are synthesized from carboxylic acids or sulfonic acids with chain lengths of   four to ten carbon atoms by substituting hydrogen atoms with fluorine atoms. Strictly speaking, PFCs can be divided into two groups: Perfluorinated alkyl-sulfonates (PFAS) among which perfluorooctanesulfonate (PFOS) is the most widely known compound and perfluorinated carboxylic acids (PFCA), whose most famous representative is perfluorooctanoic acid (PFOA). Experts are of the opinion that PFCs only have limited toxicity. As to long term effects, there is no consensus even though PFCs have been reported as having cancer promoting properties

and though the USEPA considers PFOA a "probable human carcinogen". In fact, mainly the effects of PFOA and PFOS have been investigated while those of other PFCs have been less extensively studied. Concentration limits for PFCs in drinking water The German Commission for Drinking Water lists an upper concentration limit of 1 µg/L.

Developing an automated SPE-HPLC-MS/MS method
The automated method presented in this work is based on the current ISO standard method: "ISO 25101:2009 Water quality – Determination of perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) – Method for unfiltered samples using solid phase extraction and liquid chromatography/mass spectrometry". While the ISO method focuses on PFOS and PFOA, our goal for the project reported here was to develop a simple and rugged automated SPE-

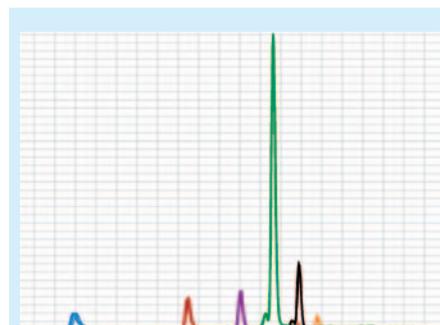## GERSTEL VENDOR SEMINAR

Thursday, July 2, 2009
14:00 - 14:45,
Conference Room 3 (KR 3)

"Improved detection limits and reproducibility in LC-MS determination of contaminants in foods and environmental samples"

Overlay MRM traces of eight different sample preparations of a waste water sample spiked with 0.5 ng/mL. Excellent reproducibility is obtained for all compounds: PFPeA (blue), PFHxA (brown), PFHpA (purple), PFOA (green), PFNA (red), PFOS (black), PFDA (yellow)

HPLC-MS/MS method for the determination of a wider list of PFCs in water and sludge. Automating the SPE process eliminates extensive and tedious manual sample preparation steps that are known sources of errors, for example when cartridges run dry or when sample matrix changes the flow of liquids through the cartridge.

The benefits of automation are manifold: Higher recovery, improved reproducibility, higher sample throughput, increased flexibility and reduced exposure of laboratory staff to potentially hazardous solvents. Automated SPE can be performed using two different setups: The SPE system can be coupled directly to the HPLC-MS/MS instrument, enabling direct sample introduction of the extracts and fully automated operation from SPE to LC-MS/MS. Alternatively, the SPE system can be operated as a sample preparation workstation separate from the analysis instruments. The WorkStation set-up provides the flexibility of choosing between different techniques or different instruments for the sample analysis in order to meet individual requirements.

Instrumentation and Methods

River water and waste water samples that had been spiked with PFCs at different concentration levels were analyzed. Method development as well as the ensuing analysis was performed using an LC-MS/MS system from Agilent Technologies (1200 Series LC and 6400 Series Triple Quad LC/MS) combined with a GERSTEL SPE system. Two SPE systems were used, one based on the single rail MultiPurpose Sampler and one based on the dual rail MPS PrepStation, which offers additional liquid handling capabilities.

The following compounds were determined using automated SPE-HPLC-MS/MS:

• **Perfluorodecanoic acid (PFDA)**
• **Perfluorononanoic acid (PFNA)**
• **Perfluorooctanoic acid (PFOA)**
• **Perfluorooctanesulfonate (PFOS)**
• **Perfluoroheptanoic acid (PFHpA)**
• **Perfluorohexanoic acid (PFHxA)**
• **Perfluoropentanoic acid (PFPeA)**

Isotopically labeled standards were used for quantitation as recommended in the ISO 25101 standard. In this work, perfluorobutanoic acid was used since this compound was not found in the samples.

HPLC Parameters

HPLC System:	Agilent 1200 SL
HPLC COlumn:	Maisch Reprosil C18HD, 50 x 2.1 mm, 3 µm
Flow:	0.3 mL/min
Eluent:	Ammonium acetate/ Methanol (MeOH)
Gradient:	0 min 20 % MeOH
	10 min 100 % MeOH
	14 min 100 % MeOH
	15 min 20 % MeOH
Analysis time:	25 min
Injection volume:	2 µL

MS Parameters

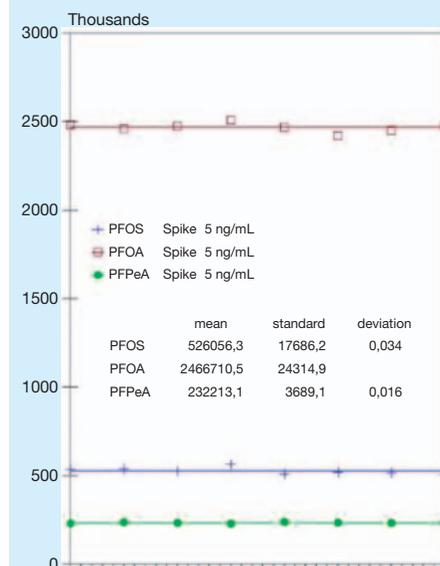|		
MSD:	Agilent 6410 Triple Quadrupole	
Ionization mode:	ESI neg.	
N_2 Temperature:	350 °C	
N_2 Flow:	10 L/min	
MS Mode:	MRM (multiple reaction monitoring)	
Mass transfers:	PFDA	513->469 m/z
	PFNA	463->419 m/z
	PFOA	413->369 m/z
	PFOS	499->99 m/z
	PFHpA	363->319 m/z
	PFHxA	313->269 m/z
	PFPeA	263->219 m/z

SPE Method

The GERSTEL MPS and GERSTEL PrepStation (Dual rail MPS) are both multi-functional autosamplers and sample preparation robots for GC/MS and LC/MS.

In addition to automated SPE using standard cartridge formats, practically all standard sample preparation techniques used for LC/MS and GC/MS can be performed automatically. This provides the user with signi-


	Correlation Coefficient
PFPeA	0,9998
PFHxA	0,9996
PFHpA	0,9997
PFOA	0,9966
PFNA	0,9993
PFDA	0,9991
PFOS	0,9992
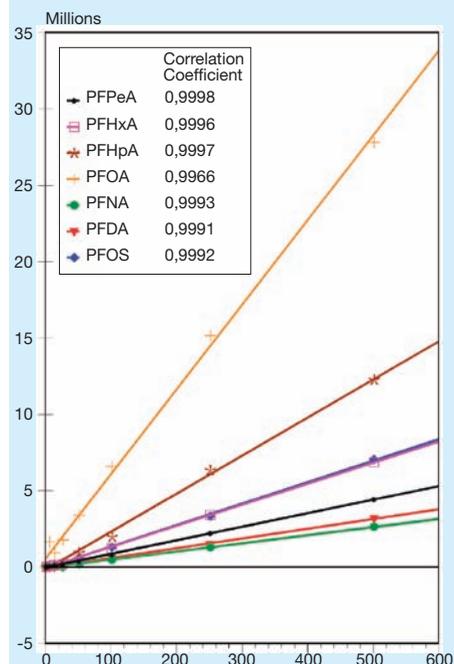
PFC calibration curves covering the range from 5 to 500 ng/mL based on external standard calibration.

		mean	standard	deviation
	PFOS	526056,3	17686,2	0,034
	PFOA	2466710,5	24314,9	0,010
	PFPeA	232213,1	3689,1	0,016

PFOS Spike 5 ng/mL
PFOA Spike 5 ng/mL
PFPeA Spike 5 ng/mL

Reproducibility of the method for PFOS, PFOA, PFPeA.

ficant added value: Method development tasks can be performed in a highly flexible manner and routine analysis chores handled efficiently and productively with minimal intervention. Among other benefits, the SPE systems are controlled through the MAESTRO software or integrated with the Agilent ChemStation and MassHunter. Just one sequence table is required to operate the entire system from SPE through liquid sample prep and sample introduction to GC/MS or LC/MS analysis. Everything is performed using just a few mouse-clicks. The PrepAhead function enables the system to plan ahead delivering time-optimized sample preparation. Samples are prepared just-in-time for introduction exactly when the LC/MS system becomes ready after the previous run, ensuring that the LC/MS is utilized to its fullest capacity. In this work, the sample preparation steps were completed in around 25 minutes.

The following diagram shows the individual steps in the SPE process as performed by the MPS- or MPS PrepStation based SPE systems. Standard format cartridges of the type Oasis WAX 150 mg 6cc were used.
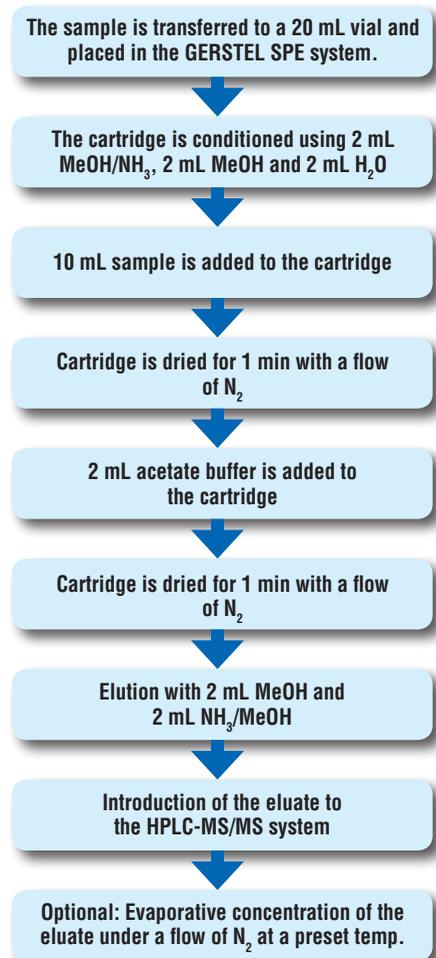
cibility results. System performance was productive, reliable and rugged,

Automated SPE is performed by the MPS or MPS PrepStation using commercially available standard volume cartridges. This means that existing manually performed SPE methods can easily be transferred to the automated system as is shown in the case of the ISO 25101 method. For every sample, a new cartridge is used eliminating the risk of cross contamination. Thanks to the PrepAhead function, LC/MS analysis of the current sample and SPE of the next sample in the sequence table are performed simultaneously, ensuring maximum productivity. For the PFC method, sample preparation took 25 minutes to complete. Apart from the time required to prepare the first sample for introduction, the LC/MS system is never slowed down by the sample preparation. Calibration based on samples spiked with 5 - 500 ng/mL of PFCs produced excellent linearity and reproducibility. Relative standard deviations were between 1 and 3 % depending on the compound. Following 2.5-fold enrichment of the SPE eluate, limits of quantitation were 0.5 ng/mL. Automated enrichment/concentration of the SPE eluate

up to a factor 100 is possible and this would lead to a further significant reduction in the limit of quantitation.

Conclusion and outlook

The automated SPE-LC/MS/MS method for determination of PFCs in water and sludge proved its worth in all aspects. The SPE process is performed using positive displacement pumping of sample and eluent, reducing matrix and restriction variation effects in the SPE cartridge in a simple and efficient manner. The automated method produces results of significantly higher quality than the ISO 25101 method in terms of both sensitivity and reproducibility, especially when analyzing difficult samples such as waste water.

The results of our work indicate that the automated method described in this work would be highly suitable for the determination of PFCs in other matrices such as urine and blood.

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The sample is transferred to a 20 mL vial and placed in the GERSTEL SPE system.

$\downarrow$

The cartridge is conditioned using 2 mL MeOH/NH$_3$, 2 mL MeOH and 2 mL H$_2$O

$\downarrow$

10 mL sample is added to the cartridge

$\downarrow$

Cartridge is dried for 1 min with a flow of N$_2$

$\downarrow$

2 mL acetate buffer is added to the cartridge

$\downarrow$

Cartridge is dried for 1 min with a flow of N$_2$

$\downarrow$

Elution with 2 mL MeOH and 2 mL NH$_3$/MeOH

$\downarrow$

Introduction of the eluate to the HPLC-MS/MS system

$\downarrow$

Optional: Evaporative concentration of the eluate under a flow of N$_2$ at a preset temp.

Results and Discussion

The automated SPE-LC-MS/MS method presented in this work resulted in excellent calibration, sensitivity, recovery and reprodu-

Pesticide analysis EZ

When the sample matrix no longer matters

Application specialists from TeLA GmbH, Germany, have developed a new method that dramatically simplifies LC/MS determination of pesticide levels, providing high-quality results independent of the sample matrix type and complexity.

The standard QuEChERS method enables rapid sample preparation for determination of pesticides in fruit and vegetables. The main benefit of this sample preparation method is that the overall analysis is less time-consuming and less error-prone than more traditional approaches. The limits of QuEChERS are encountered whenever samples with more complex matrices need to be analyzed, such as garlic, onion, artichoke or avocado with high fat content. This can lead to problems with interferences, than can especially influence quantification unless further clean-up steps are performed.

The GERSTEL SPE system provides an excellent solution, enabling reliable and rugged analysis independent of the sample matrix. The system was previously used successfully for the determination of aflatoxins, chloramphenicol and malachite green in foods (please see articles in the LC/MS special issue).

Raw sample extracts were automatically loaded onto standard SPE cartridges and cleaned. A new cartridge was used for every sample to eliminate cross-contamination. Automated SPE clean-up as described in this article took around 20 minutes to complete. Apart from the first sample, the SPE process was performed during LC/MS or GC/MS analysis of the preceding sample, ensuring that the SPE step was performed without increasing the overall analysis time.

Sample clean-up using SPE contributed not only to the ruggedness of the method, it also improves reproducibility and linearity, among other things. Orange oil samples were cleaned up using a slightly modified SPE method. Recovery for various pesticides in this difficult matrix ranged from 70 to 90 % while recoveries from fruit and vegetable samples were mainly in the range from 80 to 100 %.

A Europe-wide round robin was successfully passed. A vegetable sample (zucchini) had to be analyzed for 185 different pesticide residues. Out of 46 laboratories, TeLA using the GERSTEL SPE was among the 12 that managed to correctly identify and quantify the analytes thus meeting the round robin requirements and passing the test.

The SPE LC-MS/MS system used by the TeLA scientists for the pesticide multi-residue method consisting of an Agilent Series LC 1200 and a GERSTEL SPE system mounted over an Agilent 6410 MS/MS Triple Quad.
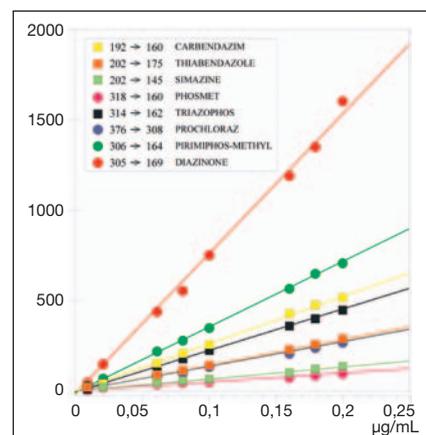
Calibration curves for nine pesticides