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HPLC 2008

GERSTEL Newsletter

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May 2008

Improving performance, selectivity and stability

Method development and sample preparation for LC and LC/MS analysis is not a trivial matter. In recent years, opinions have been voiced that samples can be injected without any or much preparation and compounds detected and quantitated without prior separation. The LC/MS or LC-MS/MS system, it was said, would handle the matter nevertheless.

In many application areas, food analysis for one, extensive sample preparation and sample clean-up is necessary in order to reach the required limits of determination and in order to keep the analysis system sufficiently stable for a routine analysis environment. Even with highly sophisticated mass spectrometers, a high level of matrix background can significantly influence system stability and produce analytical interference.

The benefits of automated sample preparation for LC and LC/MS analysis for a number of different food safety applications have been documented a collaboration between GERSTEL*, TeLA GmbH and Agilent Technologies. The following material provides an overview.

** GERSTEL develops, produces and supports solutions for LC and LC/MS based on the MultiPurpose Sampler (MPS). Techniques include automated Solid Phase Extraction (SPE) as well as Standard Addition, Derivatization, and Eluate Concentration with or without Keeper Solvent. Sample preparation is performed in parallel to LC separation for best possible productivity and system utilization, followed by sample introduction to the LC or LC/MS. The complete system is controlled by the GERSTEL MAESTRO software or through the Agilent Technologies LC/MS software.*

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 fruits 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.

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.

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.

GERSTEL VENDOR SEMINAR

Thursday, May 15, 2008,
12:45-1:45 pm, Room Kent A

Cost Effective Automated Sample Preparation with intuitive software control

❊

Automated SPE and sample preparation for improved detection limits and reproducibility in HPLC-MS determination of assorted contaminants in food

Improved determination of shell-fish toxins

Various organic compound classes are counted among marine biotoxins. Paralytic Shell Fish Poisoning (PSP) toxins, for example GTX-2, GTX-3, GTX-5 and Saxitoxin, are among the most potent biotoxins known.

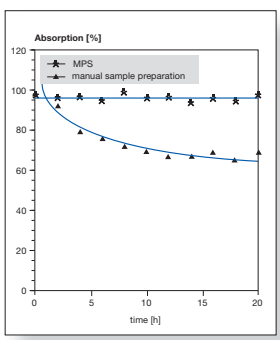
European Union (EU) guidelines require that tests for marine biotoxins be performed on a routine basis using bioassays. In the case of mussel toxins, this means performing the test on animals. Germany mainly relies on chemical analysis methods; animal experiments are used only in cases where inconclusive results have been obtained.

The analysis technique mainly used is Liquid Chromatography (LC). GTX-2, GTX-3, GTX-5 and Saxitoxin are separated on an RP column with highly sensitive fluorescence detection at the excitation wave length of 330 nm and emission wave length of 390 nm.

Depending on the detector used, the detection limit of the method lies between 5 µg/kg and 50 µg/kg mussel meat. GTX-2 and GTX-3 are not separated when using pre-column derivatization scince they form the same product. A post-column derivatization method is required in order to determine these compounds individually.

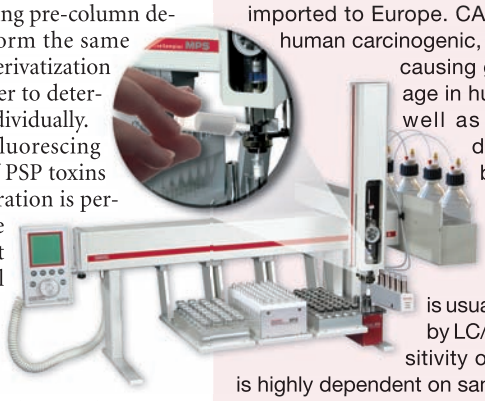
The problem is that fluorescing derivatization products of PSP toxins are labile. If sample preparation is performed manually and the samples subsequently left in the autosampler until analyzed, a significant decrease in signal intensity is found due to decomposition of the oxidation product. As an example, a 30 % drop in the concentration of GTX-5 is observed after 12 hours at 25 °C.

If sample preparation is performed ontime by the GERSTEL MPS for every sample, the determined concentrations remain constant over many hours. The MPS can prepare a sample during analysis of the preceding sample, saving time and optimizing system utilization while delivering significantly improved results.



Comparison data for manual and automated sample preparation: Analyte degradation is clearly observed in samples that have been prepared manually and left on the autosampler for more than two hours prior to sample introduction and analysis. The MPS prepares every sample at the exact same point in time prior to analysis, providing significantly better results.

Analyzing Chloramphenicol in half the time



The antiobiotic Chloramphenicol (CAP) is banned for use in food products of animal origin such as meat and fish that are imported to Europe. CAP is a known human carcinogenic, suspected of causing genetic damage in human cells as well as irreversible damage to the blood-forming cells of the bone marrow. The determination of CAP is usually performed by LC/MS. The sensitivity of the method is highly dependent on sample preparation. A high matrix load can result in incorrect quantification of CAP even when highly selective LC-MS/MS methods are used. The same applies to many other analytes in the areas of food, pharmaceutical or environmental analysis. SPE (photo) is the sample preparation technique of choice for many such samples, separating analytes from the matrix prior to LC or LC/MS determination. Manual SPE methods have serious drawbacks: A lot of time and patience is needed and recovery and reproducibility can be subject to extreme deviations, largely depending on the experience and diligence of the user. Using an automated SPE-LC/MS system for the determination of CAP in food products of animal origin provides standard deviations in the order of 2.0 % with a recovery of 92 %. Automating the SPE process and associated liquid handling steps provides improved reliability, productivity and throughput.

Fast and reliable answers regarding aflatoxins in foods

Mycotoxins, particularly the aflatoxins B1, B2, G1 and G2, can lead to acute illness as well as chronic aliments, caused by carcinogenic, mutagenic and hormone active properties. The method of choice for reliable and sensitive determination of aflatoxins is Solid Phase Extraction (SPE) or affinity chromatography, combined with LC/MS analysis.

Established sample preparation methods used in LC/MS determination of aflatoxin levels provide only limited scope for optimization, but reliable and useful analysis results can be obtained in less than half the time if the SPE process is automated.

The GERSTEL SPE solution automates all steps from standard addition and derivatization trough Solid Phase Extraction to LC/MS analysis. Software-controlled parallel processing of sample preparation and analysis reduces analyte decomposition. The preparation steps for each sample are performed at exactly the same point in time prior to analysis for best possible results.

Malachite green in fish products

Malachite green (MG), a toxic fungicide used in fish farming, as well as its main metabolite leuco malachite green (LMG), are easily ionized using electrospray ionization in positive ion mode. In contrast to MG itself, the metabolite forms a doubly charged ion (m/z 166) in addition to the singly charged molecular ion $[M+H]^+$. In MS^2 mode of the ion trap the MG precursor ion forms a product ion (m/z 313), while the doubly charged LGM precursor forms a fragment, which is also doubly charged. This transition is highly selective and can be used for extremely sensitive determination of leuco malachite green.

Using these two transitions, it was possible to reach limits of determination of 0.5 µg/kg for malachite green and 0.05 µg/kg for leuco-malachite green.

Automated Solid Phase Extraction (SPE) directly coupled to the LC/MS system provided a combination of high recovery rates, in excess of 90%, and excellent reproducibility of the sample preparation step. The time required for sample preparation was reduced by approximately 50%, enabling a significant increase in throughput and laboratory productivity.