MatrixEX™ - A Fully Automated Column Switching System to Reduce Matrix Effects in LC-MS/MS Analysis

An Example of using the MatrixEX™ System for Pesticide Screening in Complex Food Samples

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Overview

A dream of analytical chemists is to have tools at hand to analyze high volumes of samples faster, but without sacrificing performance. An ever bigger dream is if this process were automated to even further improve efficiency and simplicity.

Is it possible?

Yes. Here we present a new workflow utilizing automated LC column switching to improve and enhance development of both single and multi-residue pesticide analysis in complex matrices. MatrixEx™ is an innovative and a completely automated column switching system for LC-MS/MS allowing:

- Increased throughput of samples by employing up to nine different LC columns
- Increased robustness and analytical performance with the implementation of back-flushing the separation column for efficient and complete removal of residual matrix components using alternating chromatographic conditions and two identical LC columns
- Enhanced simplicity with a fully automated system for flexible column and eluent selection
- Universal use for food, environmental, and metabolomics applications

Figure 1. The MatrixEx™ column switching system

Introduction

The continuous improvement of the quality, performance, and efficiency in chemical residue analytical methods is constantly necessary to cope with the increasing demands in food safety and consumer protection.

Nowadays, complex analytical techniques such as GC-MS(/MS) or LC-MS/MS are established in most residue laboratories. Triple quadrupole mass analyzers became popular for targeted screening and quantitation in residue analysis because of their high selectivity and sensitivity when operated in Multiple Reaction Monitoring (MRM) mode.

The recent progress in speed, sensitivity, and robustness of LC-MS/MS instrumentation has allowed a simplification in sample preparation and a significant reduction in chromatographic run times while still enabling laboratories to detect hundreds of chemical residues, such as pesticides, at trace levels with very high reproducibility and confidence.

However, generic sample extraction and shortened chromatographic separation can result in co-elution of target analytes and matrix components, leading to interferences during

ionization using Electrospray Ionization (ESI). These matrix effects often result in ion suppression or enhancement of the target analytes, and can lead to highly inaccurate quantitation results.[1-3]

Furthermore, these co-extracted matrix components can also collect on the LC column. Even after long column flushes with organic solvent, these lingering matrix components may result in unexpected and irreproducible matrix effects in subsequent chromatograms.[4]

Finally, comprehensive food residue analysis requires a diverse array of multi-residue methods and single-residue methods, many of which require very different LC analysis conditions. Thus, the ability to automatically switch between multiple columns and different mobile phases in a single analytical run is desirable for high throughput food testing laboratories.[5]

The development of automated column switching systems enables the use of alternating chromatographic conditions and free column selection to run multi-residue and single-residue methods on one single LC-MS/MS instrument. Because the column and mobile phases do not have to be changed manually, this design greatly increases a laboratory's sample throughput productivity.

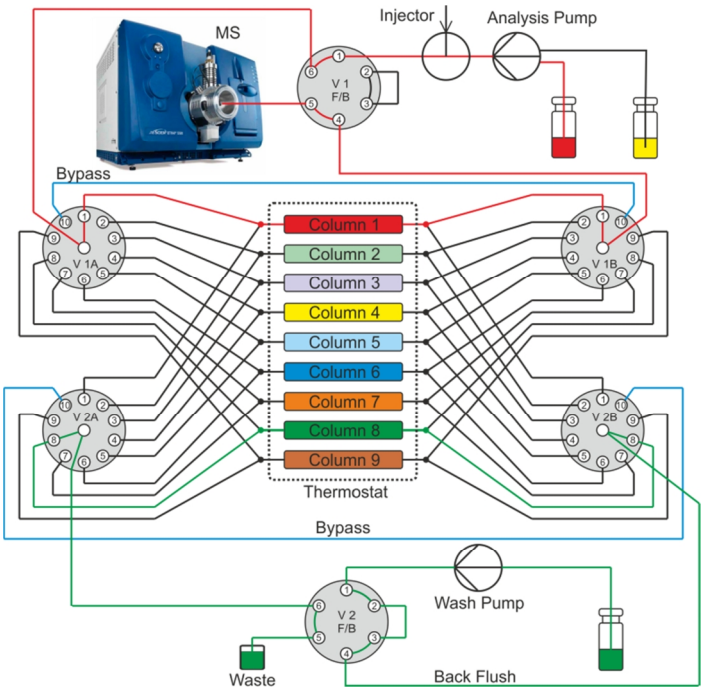

Figure 2. Schematic for a fully automated column switching system as used in the MatrixEX™ system

Furthermore, the column switching system allows directional changes of the flow of the mobile phase through the column. The ability to reverse the flow enables an automated column flushing procedure to quickly and efficiently remove residual matrix components from the LC column. The result is decreased matrix effects during the analysis, improved life of the LC analytical columns, and higher quality quantitative data.

Experimental

Samples and Sample Preparation

Different food samples were extracted using a QuEChERS procedure and analyzed for over 250 pesticides after dilution.

LC-MS/MS Conditions

An Agilent LC system containing a binary and an isocratic pump and autosampler was connected to the MatrixEX™ system (MayLab Analytical Instruments GmbH, Vienna, Austria).

The SCIEX QTRAP® 5500 system equipped with Turbo V™ source and ESI probe and operated in MRM mode using the *Scheduled* MRM™ algorithm was used for the analysis. Three methods were multiplexed on the system for analysis in a single sequence:

1. Multi-pesticide screening for approximately 250 pesticides:
   - Monitoring of >500 MRM transitions to identify and quantify pesticides using the ratio of quantifier and qualifier ion
   - LC separation using a Phenomenex Kinetex C18 (100 x 2.1 mm; 2.6 µm) column and a mobile phase containing water/methanol and 5mM ammonium formate
2. Single-residue method for polar pesticides (Ethephon):
   - LC separation using a Hypercarb (100 x 2.1 mm, 5 µm) column and a mobile phase containing water/methanol and 1% acetic acid
3. Single-residue method for polar pesticides (Chlormequat, Mepiquat):
   - LC separation using a HILIC (150 x 2 mm, 5 µm) column and a mobile phase containing water/acetonitrile and 25 mM ammonium acetate

An AAO driver for Analyst® software developed by MayLab controls the MatrixEx™ switching system and is fully integrated in the Analyst sequence of the analytical procedures.

Results and Discussion

The MatrixEx™ system (Figure 1) combines a unique, fully automated, and patented setup to select from up to 9 columns (plus bypass) for LC-MS/MS using different LC methods under optimal mobile phase conditions.[6]

This switching scheme of the MatrixEX™ system is shown in Figure 2.

When running two columns in parallel, the direction of the LC flow can be selected accordingly. This allows the analyst to rinse one analytical column under reverse flow to efficiently wash away matrix components from previous injections while simultaneously performing an analysis on a different column. This feature not only improves throughput and capacity on a single LC-MS/MS system but also helps to reduce matrix effects in MS/MS detection in the next analytical run, increasing the robustness of the analytical method.

Using the MatrixEx™ System Significantly Decreases Matrix Effects and Increases Robustness

Matrix effects were measured by post-column infusion of a standard while running sample extracts on the LC-MS/MS system (Figure 3).

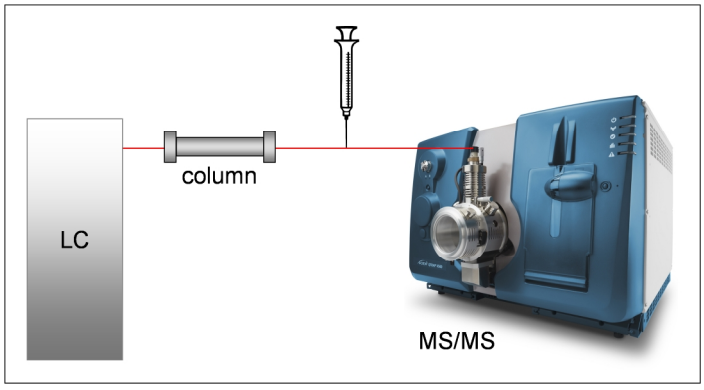

Figure 3. Post-column infusion of a standard to measure matrix effects

The intensity of MRM signal of the infused standard was monitored and used to create a profile of the matrix effects, normalized to a reference signal free of matrix compounds (i.e. after solvent injection).[1]

The result of this procedure revealed that:

- Ion suppression can be as high as 80% or more.
- Matrix components from previous injections are still retained on the column and result in unexpected and irreproducible detection effects in the subsequent chromatograms.
- Extended washing of the column with organic solvent in direct direction is not sufficient to remove all matrix components from the column before the next injection (Figure 4).[4, 7]

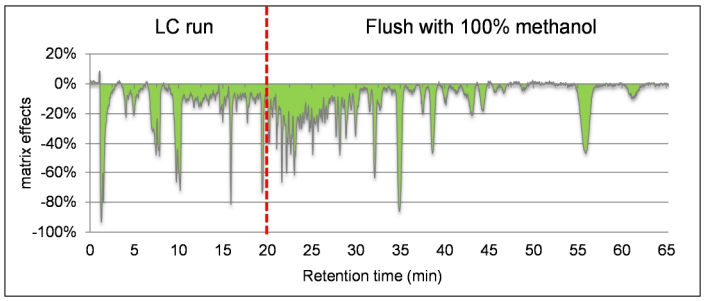

Figure 4. Profile of matrix effects caused by an extract of Arugula sample; to completely remove matrix components from the column, back-flushing should be applied immediately after the last analyte has eluted (i.e. after 20 min).

The MatrixEx™ system now allows fully automated column switching and the concurrent use of a selected pair of LC columns, allowing users to inject a sample onto one column for chromatographic separation while simultaneously flushing a second column after a run had been finished. Ideally, the back-flush can be started when the last analyte has been detected (see Figure 4, at ~20 min). The complete removal of matrix components by back-flushing sometimes requires the use a higher amount of organic mobile phase the column than for the chromatographic separation itself. The MatrixEx™ system enables this by allowing multiple solvent reservoirs to be used interchangeably across different LC columns on the multiplexed system.

This procedure guarantees that matrix components from previous analyses do not affect the quantitative results of later runs, ultimately increasing reproducibility. Figure 5 shows the repeatability of peak areas when analyzing hop extract samples. The relative standard deviation of results after back-flushing was as low as 1.0%, compared to 8.6%, without the back-flushing procedure.[6]

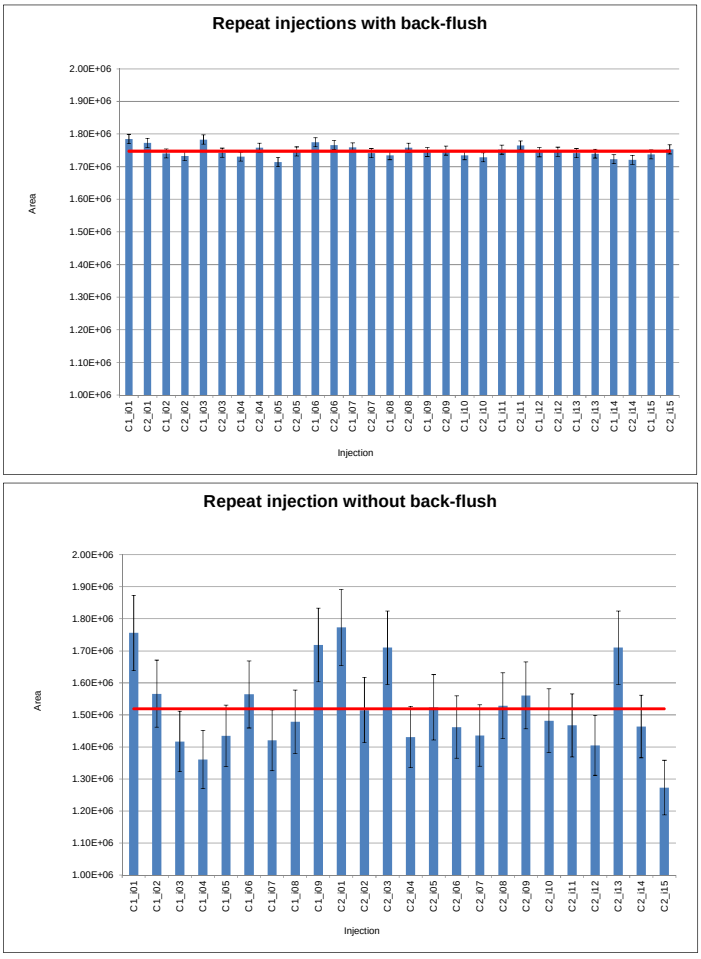

Figure 5. Reproducibility of quantitative results with back-flush (top) and without back-flush of the column (bottom)

In addition, the continuous efficient removal of matrix from the column dramatically increases the longevity of the LC column. Figure 6 shows the reproducibility of retention times over a period of more than 5 months (>8000 injections).

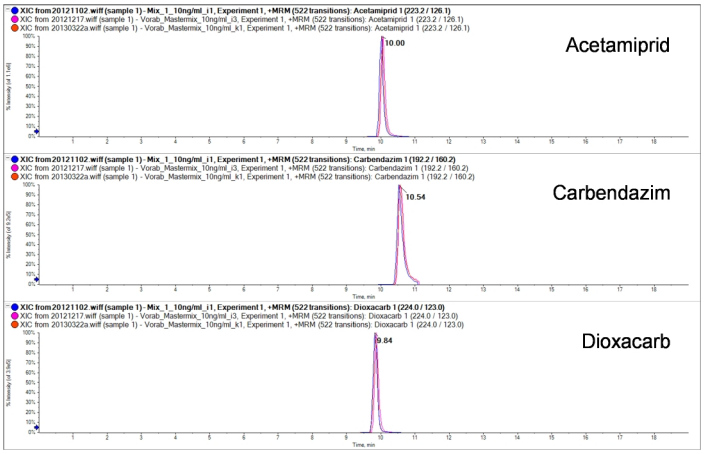

Figure 6. Reproducibility of retention times of three selected pesticides over a period of more than 5 months, blue: injection #10, purple: injection #3000, orange: injection #8000

Using the MatrixEx™ System to Switch LC Conditions

The MatrixEx™ system allows a fully automated setup to select two chromatographic columns out of up to 9 columns with different mobile phases for LC-MS/MS analysis. This means users are able to select a pair of columns for each method from up to 4 different LC methods while also providing the above described column back-flush procedure to reach the best quantitative results and robustness of the system.

An example for multiplexing three different residue methods using a reversed phase, Hypercarb, and HILIC column is shown in Figure 7.

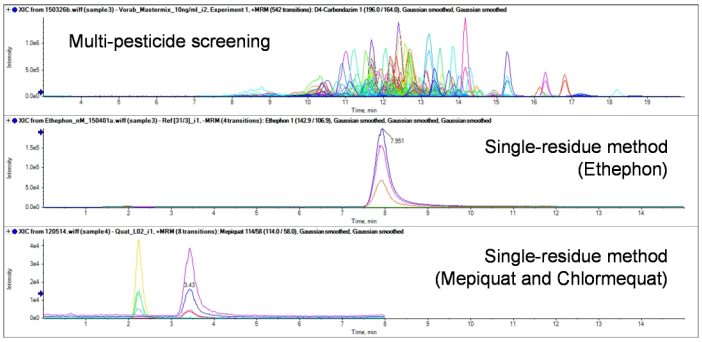

Figure 7. Example chromatograms for multi-pesticide screening and two different single residue methods, the switching of columns and mobile phase was done automatically using the MatrixEX™ system

Column switching, mobile phase selection, and column back-flush is fully software-controlled through an AAO driver developed by MayLab, offering complete automation and implementation in a routine testing laboratory. The screenshot of this software is shown in Figure 8.

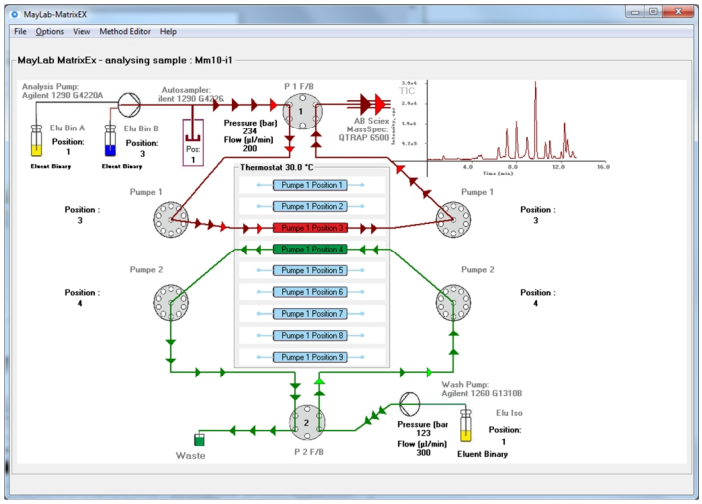

Figure 8. Screenshot of the AAO driver to control the MatrixEX™ system

Summary

The presented fully automated column switching system, MatrixEX™, offers routine testing laboratories a new innovation in LC-MS/MS analysis to reduce matrix effects, improve throughput, improve quality of analytical results, and potentially reduce costs in consumable products:

- Reduced matrix effects by automated column-flushing between LC-MS/MS runs
- More accurate and reproducible quantitation and improved robustness from reduced matrix effects
- Increased sample throughput by simultaneous multiplexing of multiple different methods on a single LC-MS/MS system
- Extended lifetimes of the LC columns

In addition, the system allows the independent selection of up to 9 LC columns (plus bypass) and different optimized mobile phases to apply different LC conditions for analysis in combination with the AAO driver for the Analyst® software for complete automation. Optionally, EluSwitch, a mobile phase selection valve, is available for the isocratic pump.

The described MatrixEX™ system is in routine use at the WESSLING Group (Berlin, Germany) since 2011.

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For additional product information visit the website of MayLab Analytical Instruments GmbH (Vianna, Austria).

http://www.maylab.info/index.php/products/MatrixEX

http://www.maylab.info/index.php/products/EluSwitch

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