Multiplexing Two Different Food Residue Methods using HILIC and Reversed Phase Chromatography in the Same LC-MS/MS Run

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Overview

Multiplexing liquid chromatography (LC) systems, and synchronizing to a single tandem mass spectrometer (MS/MS), can generate the high throughput needed by maximizing the efficiency of the MS/MS detector. An integrated multiplexing system has been specifically designed to synchronize two LC systems and an AB SCIEX mass spectrometer, allowing injection of samples into two LC streams in parallel. The overlapping LC runs and efficient use of MS detection, achievable with the AB SCIEX MPX™-2 High Throughput system, is shown to result in an overall higher throughput for common routine analyses.

Here two applications are presented to analyze milk extracts for chemical residues in the same LC-MS/MS run. The applications are examples of two opposite extremes usage in mobile phase. The first stream was used to implement an antibiotic screening with reversed phase (RP) setup while the second stream was used to analyze melamine with a hydrophilic interaction chromatography (HILIC) setup. The MPX™-2 High Throughput System can be easily configured and controlled to multiplex the two different analyses in the same run and achieve an overall higher throughput for common routine analyses.

Introduction

The incidences regarding the determination of melamine and cyanuric acid in wheat gluten as the cause of animal deaths and more recently hospitalization of thousands of children as a result of consumption of melamine contaminated milk products have highlighted the need for accurate analytical techniques to quantify and identify melamine in food. This is to allow manufacturers and regulatory agencies to pro-actively ensure consumer product safety. With the fast growing numbers of food items to analyze and having to maintain the fast turnaround times, so as to minimize the cost impact to manufacturers, a system is required to increase throughput of the analysis.

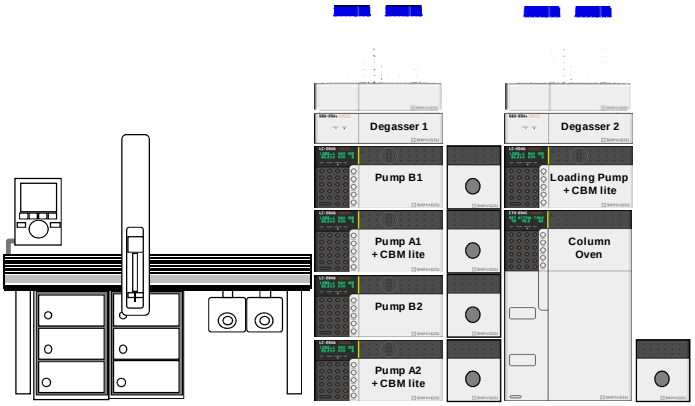

Figure 1. AB SCIEX MPX™-2 SP High Throughput system: configuration and module arrangement

HILIC separation with MS/MS detection is a fast, selective, and sensitive method for the analysis of Melamine in food samples after simple extraction.[1-3]

Antibiotics are used against infectious diseases with great success and are part of modern agriculture for many years. The beta-lactam, macrolide, sulfonamide, tetracycline, and other antibiotics help to maintain the health of the animal. However, the residues of antibiotics remain in animal-derived human foods may pose potential human health hazards. In addition, the widespread use of antibiotics has resulted in the emergence of drug-resistant bacteria.

Many countries have built a series of regulations to the use, dosage, and withdrawal times for many of these antibiotics in animal production. While there are several methods to determine antibiotic residues, LC-MS/MS using RP conditions is used more widely because of its higher specificity and sensitivity, which lead to better detection and identification. [4-6]

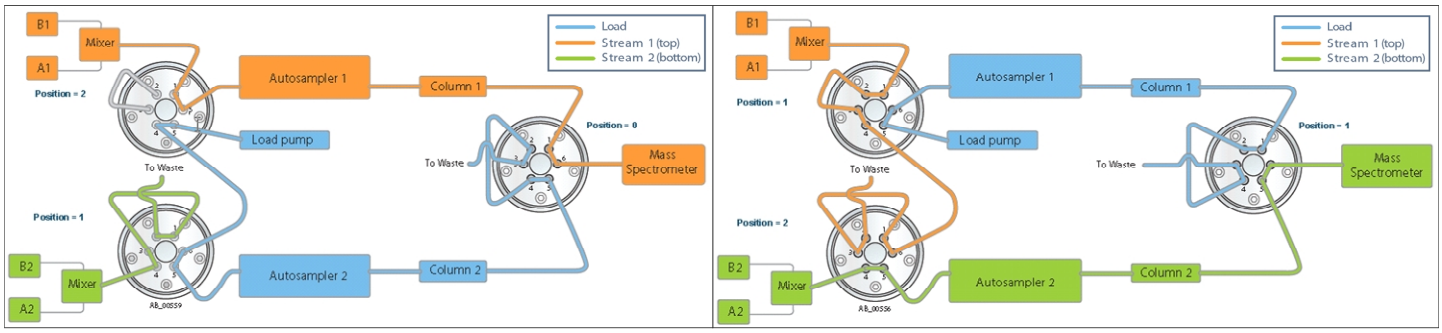

Figure 2. Solvent flow paths at typical states of a multiplex run (left: Stream 1 into MS/MS, right: Stream 2 into MS/MS)

HILIC is a chromatographic technique used for the separation of polar and hydrophilic compounds. As opposed to RP chromatography in which the stationary phase is non-polar in nature; the column packing that is used for HILIC separations is very polar. This requires the use of high organic mobile phase composition in HILIC separations, the opposite of the required starting conditions of an RP setup.

Here we multiplex a screening method for seven classes of antibiotics, using RP chromatography and a HILIC method to test for melamine using the AB SCIEX MPX™-2 High Throughput system to demonstrate the ability of the system to not only maximize throughput of two different analyses that differ in both run time and mobile phase conditions while maintaining data integrity for both analyses.

Experimental

Multiplex Hardware Setup

An integrated multiplex LC-MS/MS system was used consisting of an AB SCIEX 4000 QTRAP® system, two Shimadzu UFLC$_{XR}$ LC systems, a CTC PAL autosampler with DLW (dynamic load and wash) option, a pump containing a four solvent selection valve for sample loading and 5 switching valves for flow path control (Figure 1). The two chromatographic channels were not independent as they share a single high pressure loading pump which provides additional flexibility for injection and loading solvent composition.

All hardware modules were controlled by Analyst® software 1.5.1 with MPX® driver 1.1 add-on. The MPX™ driver was designed to control a two-stream LC system in various configurations in combination with any AB SCIEX mass spectrometer. It synchronizes sample injections in staggered LC runs, allows the user to create multiplex LC method, and enables targeted MS/MS data acquisition in a pre-determined retention time window using the parallel LC streams. Precise timing for the switching valves allows each LC stream to perform interleaved injection and LC gradient elution. Figure 2 shows common flow paths for a typical LC-MS/MS analysis.

Multiplex Software Configuration and Operation

After running the multiplex software installer, the user may easily configure and activate the multiplex option from the 'Hardware Configuration' dialog within Analyst® software. The MPX® driver provides an easy-to-use interface to modify system configuration in the 'Settings Pane' (Figure 3 A), to create or update multiplex LC methods in the 'Method Pane' (Figure 3 B), or to monitor the real-time acquisition and system status (including pressure, flow rate, temperature, flow path, and system state for both LC streams) in the 'Status Pane' (Figure 3 C).

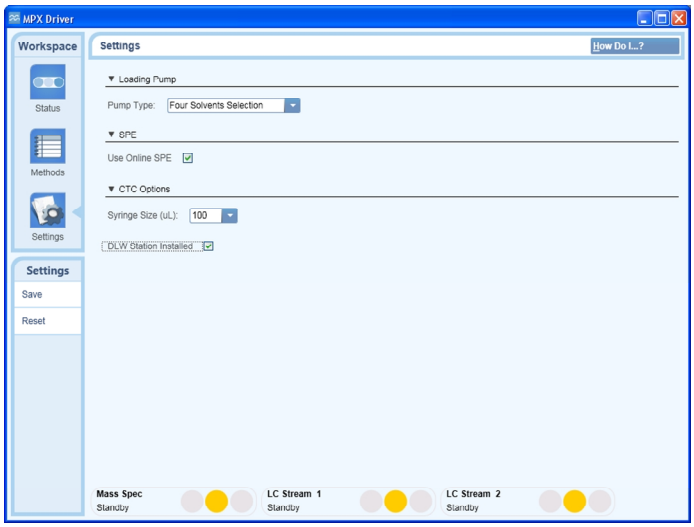

Figure 3 A. Settings Pane to modify system configuration

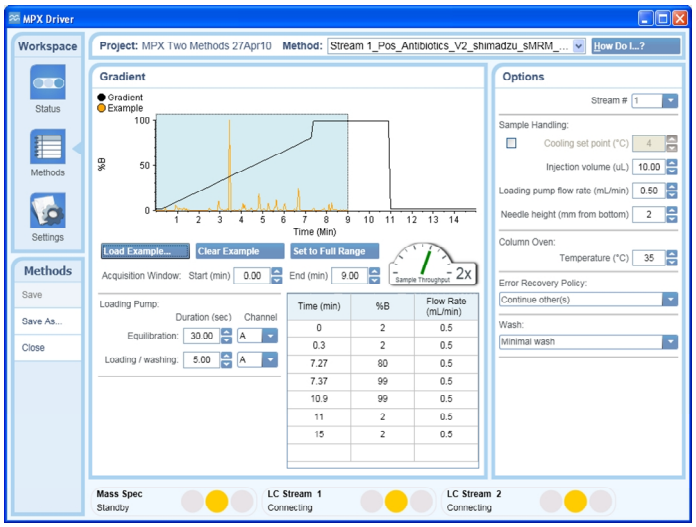

Figure 3 B. Method Pane to create or update multiplex LC methods, the blue shaded area in the chromatogram defines the MS/MS acquisition window

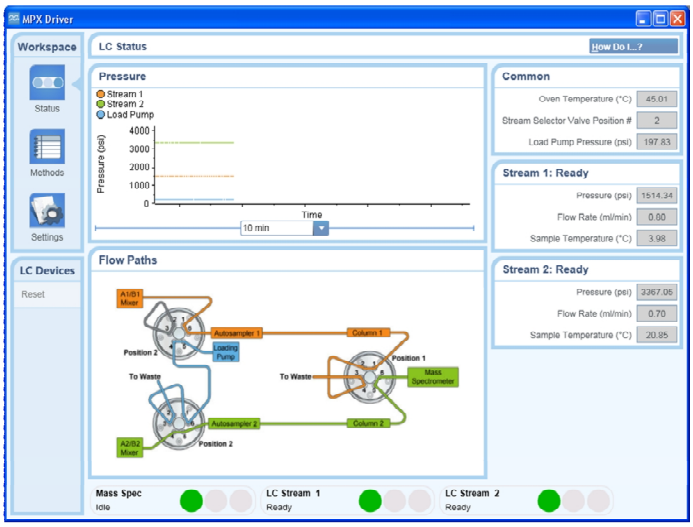

Figure 3 C. Status Pane to monitor the real-time acquisition and system status

Automated multiplex data acquisition for a batch of samples is very similar to performing a regular LC-MS/MS acquisition by creating and submitting a batch in either Analyst® or Cliquid® software. During a multiplex run, as illustrated in Figure 4, a staggered injection timing schedule is calculated in real-time by the MPX® driver. All switching valves and synchronization between LC components and the mass spectrometer are completely controlled by the MPX® driver.

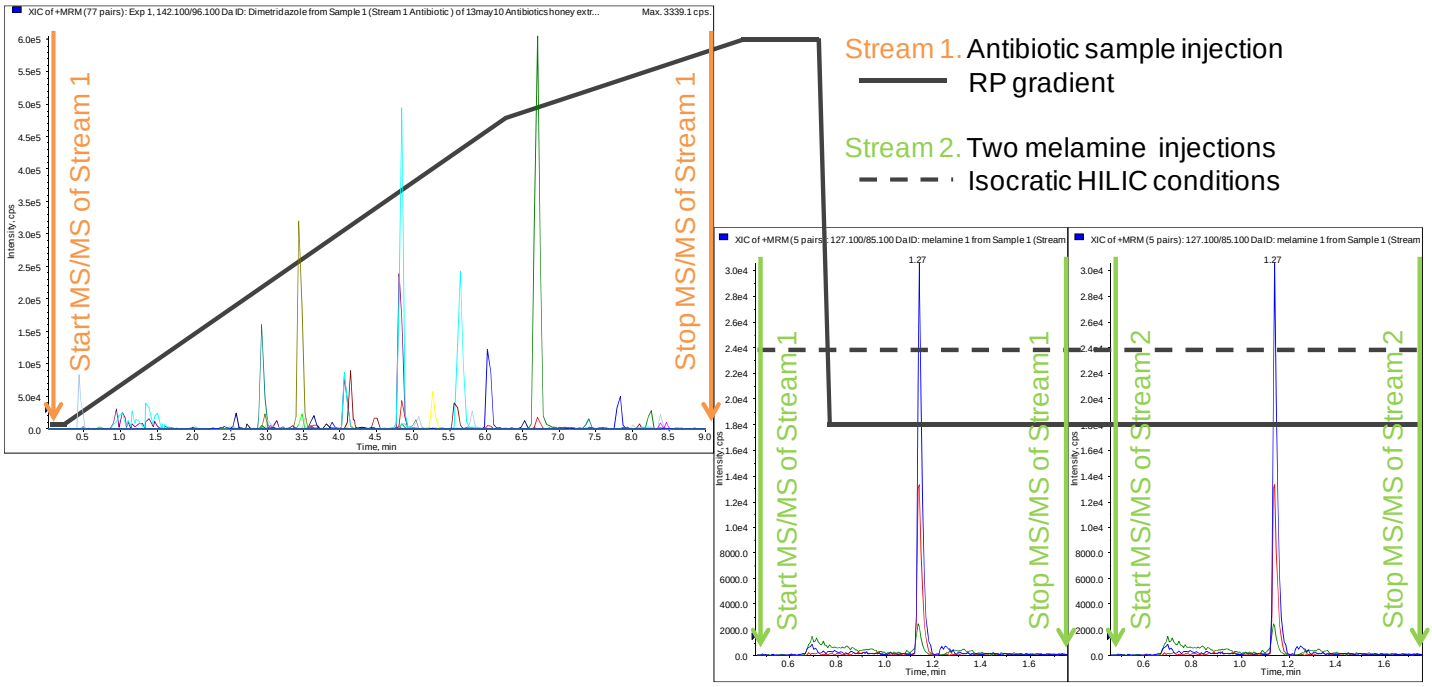

Figure 4. Multiplexing two methods that differ in their LC run times and chromatographic conditions

Results and Discussion

The AB SCIEX MPX™-2 High Throughput system has been designed to support two parallel LC streams into a single MS/MS. This arrangement allows minimizing MS/MS redundancy time during LC column equilibration and dead volume and improve throughput by as much as 2 times.

The MPX™-2 High Throughput system uses a loading pump consisting of a four solvent selection valve. The inclusion of the valve allows the flexibility of choosing between four different solvents in which to load the sample onto the column. As this loading valve is shared between the two streams, different analyses can be run on opposite streams. The system is therefore capable of multiplexing two analyses that differ in their LC run times and mobile phase conditions in the same run. An example in which such a workflow is effective is when a method with a long equilibration time, in which no analytes are eluting, is multiplexed with a method that has a short LC run time. To demonstrate this, an RP method (antibiotic screening) and a HILIC method (melamine quantitation) were multiplexed.

The antibiotic method used a non-polar C18 stationary phase (Phenomenex Gemini 3u C18 110 Á, 100x2 mm) with a polar mobile phase (water/methanol + 0.1% formic acid) as opposed to the HILIC method in which the stationary phase is very polar (Phenomenex Luna 3u HILIC 200 Á, 100x2 mm) with a less polar mobile phase (acetonitrile/water + 50 mM ammonium formate (80/20) acidified with HCl to pH 3.2). Multiplexing these two methods therefore resulted in switching quickly between solvents of wide differences in organic content. This had to be done while still maintaining chromatographic performance and data integrity for both analyses.

Antibiotic Screening

The antibiotic screening method was used to screen for a total of 77 compounds of seven classes of antibiotics, including beta-lactam, tetracycline, sulfonamide, macrolide, amphenicol, fluoroquinolone, and flunixin, in milk extracts.

The method consisted of a Multiple Reaction Monitoring (MRM) survey to automatically trigger Enhanced Product Ion (EPI) scans to identify each analyte based on their molecular fingerprint with high confidence. An example of the workflow is shown in Figure 5 which shows an MRM chromatogram and an example EPI spectrum generated from a spiked milk sample during the multiplexing of the antibiotics screening method with the melamine quantitation method.

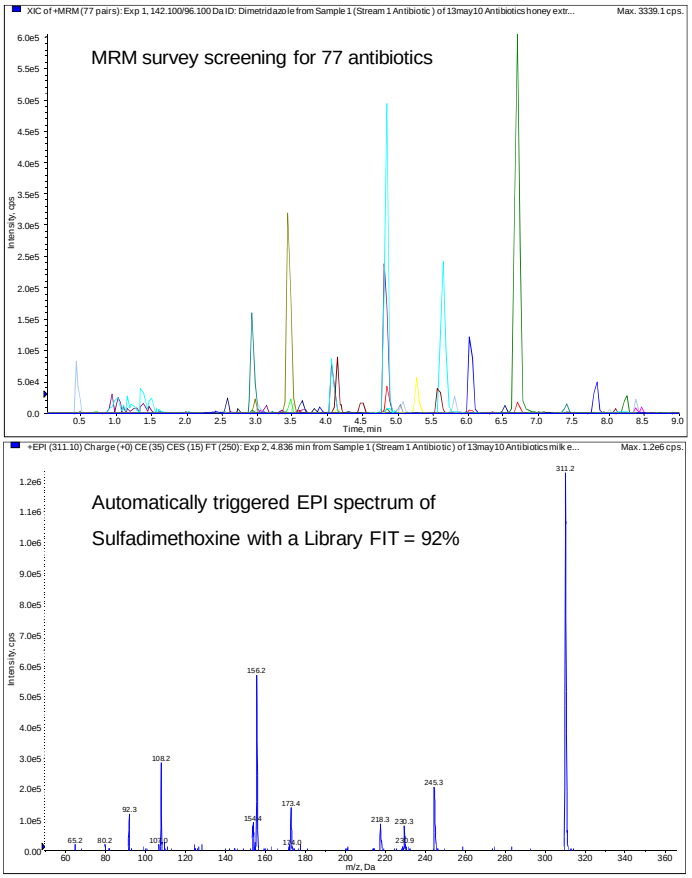

Figure 5. MRM survey and example EPI spectrum of the antibiotic screening which was multiplexed with the melamine quantitation method

Melamine Quantitation and Identification

During the same LC-MS/MS run samples were also tested for melamine. A number of three MRM transitions were monitored to quantify melamine and to perform compound identification based on MRM ratio calculation (Figure 6). The generated calibration curves highlight that the analytical performance was not compromised by multiplexing this methods with the antibiotic screening using the MPX™-2 High Throughput system (Figure 7 and Table 1).

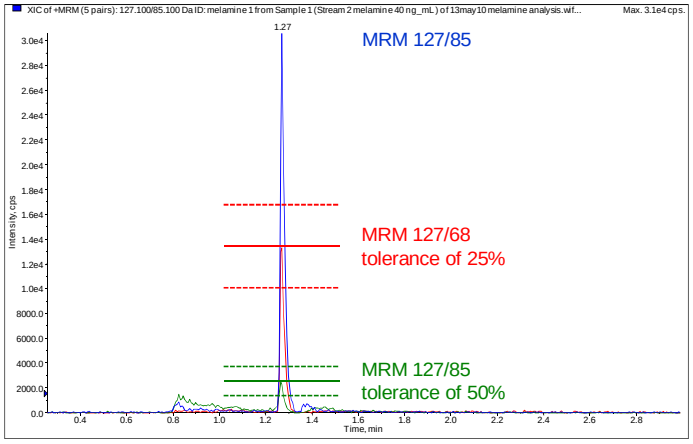

Figure 6. Chromatogram of melamine spiked into milk with MRM ratio tolerances for compound identification as defined by the European guideline 2002/657/EC[7]

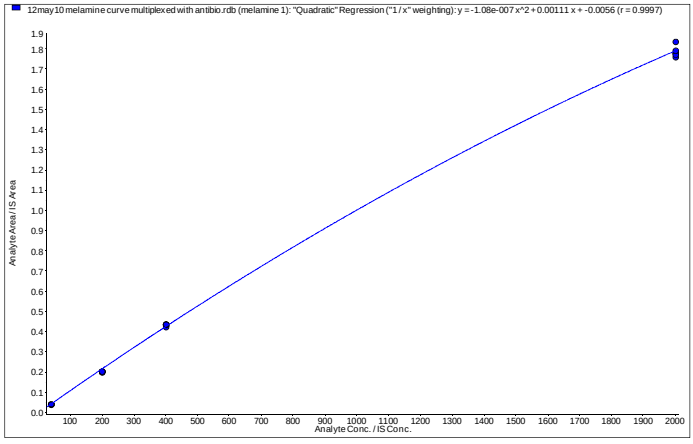

Figure 7. Calibration curve of melamine spiked into a blank milk with an r^2 of 0.9997 after quadratic regression with 1/x weighting

Table 1. Accuracy and reproducibility of quantifying melamine in milk

Concentration (ng/mL)	% CV	Accuracy in %
40	1.96	102.4
200	0.97	95.2
400	1.12	102.5
2000	1.94	99.9

Summary

Multiplexing LC systems, and synchronizing to a single MS/MS, can generate the high throughput needed by modern day laboratories to analyze increasing numbers of samples.

The AB SCIEX MPX™-2 High Throughput system has the capability of multiplexing two analyses that differ in their LC run times and mobile phase conditions in the same run and achieve an overall higher throughput system for common routine analyses. This allows for considerable time savings for analytical runs.

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Publication number: 1550210-01

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