

Advances in Automation of Food-related Analysis and Screening

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Overview

Purpose: To introduce new sample preparation methods aimed to solve formidable analytical challenges in the food-related analysis area.

Methods: Automated online sample preparation using Thermo Scientific TurboFlow technology coupled with Thermo Scientific mass spectrometers.

Results: Through using this technology for food analysis, the need for manual sample preparation is removed, resulting in significantly increased sample throughput .

Introduction

Worldwide food safety concerns have risen dramatically as the number of food contamination incidents and product recalls has increased. Therefore, accurate monitoring of contaminant levels in food and agricultural products is essential to assure the safety of the food supply and to manage human health risks. It is well-known that the basic analytical requirements in food analysis are high resolution, high throughput, high sensitivity detection and quantification of contaminants at or below the maximum residue limit (MRL) or tolerance of the compound in a given food matrix. Liquid chromatography-mass spectrometry (LC/MS) as the central enabling technology has been recognized as an indispensable tool in food safety and quality control fields[1]. LC/MS provides high speed, high resolution and high sensitivity separation of various chemical compounds.

Every food analysis starts with sample preparation, which is widely accepted as one of the most critical steps of LC/MS. Increased demand for higher throughput, accuracy and lower matrix interference from food analysis laboratories has made sample preparation the largest bottleneck. Currently, solvent extraction and solid phase extraction (SPE) are two of the most widely used methods to isolate and/or enrich target analytes from food matrices. When done manually, these offline techniques are often labor-intensive, time-consuming and costly, resulting in low sample throughput. Turbulent flow chromatography technology can eliminate the need for lengthy offline sample preparation steps, thereby eliminating these disadvantages.

Methods

Turbulent flow chromatography

This study will review a number of key applications in food safety using turbulent flow chromatography. All experiments used a Thermo Scientific Transcend TLX system powered by TurboFlow™ technology to separate analytes from various matrices prior to MS/MS analysis. The system injected the sample directly onto a narrow diameter (0.5 or 1.0 mm), TurboFlow chromatography column packed with large particles [Figure 1(A)]. High linear velocities are created inside the column, which force large molecules to quickly flow through to waste while retaining the small molecule analytes. The technology is an improvement over traditional SPE because it utilizes reusable extraction columns in a closed system, reducing the time required for offline sample preparation from hours to minutes. It also allows automatic removal of proteins and larger molecules in complex mixtures by combining turbulence, diffusion and chemistry. Figure 1(B) shows the typical configuration of a single-channel Transcend™ TLX system.

By directly injecting food samples into the LC/MS system, food safety and quality laboratories can achieve significant analytical improvements by eliminating time-consuming, costly sample preparation steps. Turbulent flow chromatography technology also allows the broad selection of stationary phases. These features make the technology a versatile and important tool in the food safety area.

FIGURE 1. Fluid Path in turbulent flow chromatography column and LC/MS system configuration

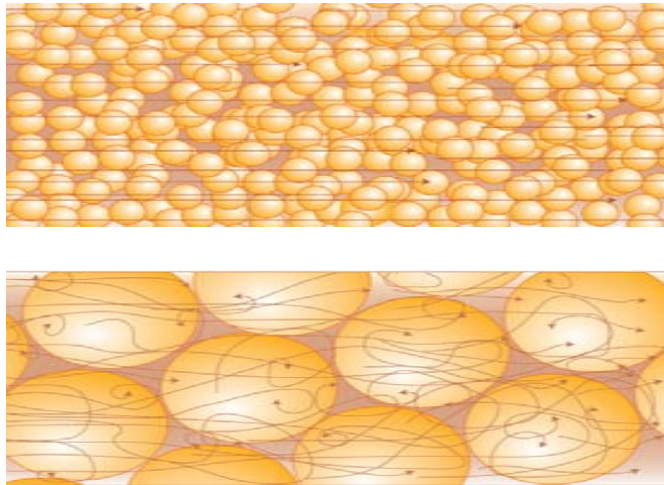


Figure 1(A)

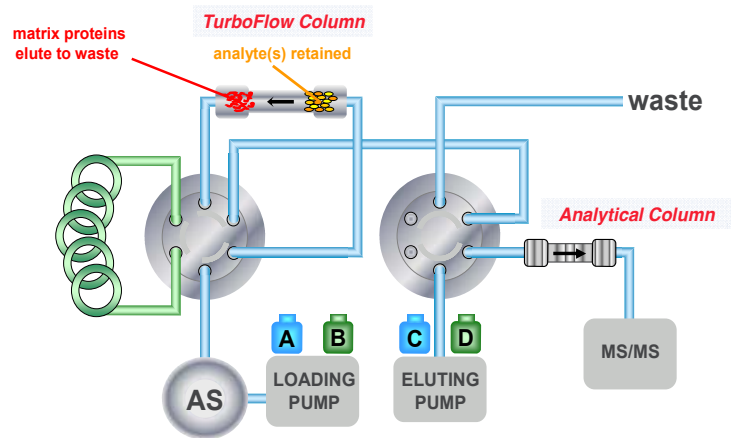


Figure 1(B)

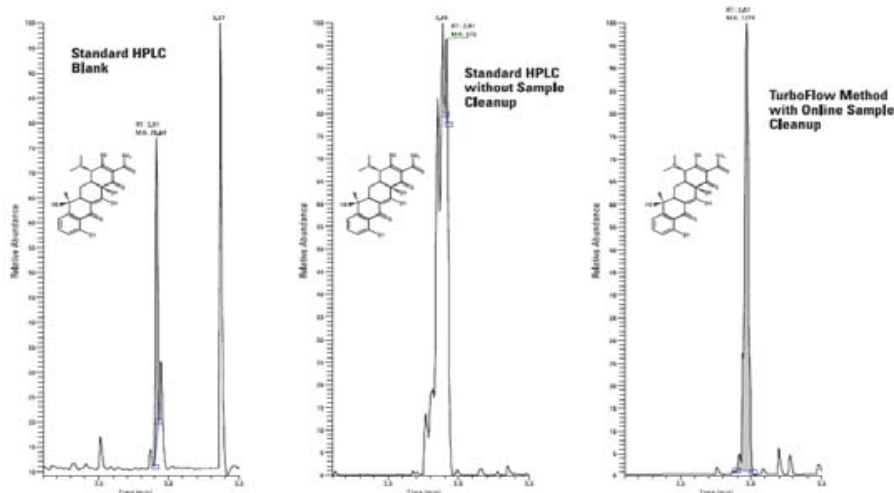
Results and Discussion

1) Veterinary drugs and chemicals

Four common chemical residues, malachite green (MG), leucomalachite green (LMG), ciprofloxacin and tetracycline in fish, shrimp and pig liver were analyzed using a Thermo Scientific TSQ Quantum Access triple stage quadrupole mass spectrometer[2]. The MRLs for these analytes range from 2 $\mu\text{g}/\text{kg}$ for the sum of MG and LMG residues in fish muscle to 100 $\mu\text{g}/\text{kg}$ for both ciprofloxacin and tetracycline in muscle for all food-producing species.

The total offline sample preparation time was approximately 30 to 40 minutes, including homogenization, centrifugation and calibrator preparation. Figure 2 compares representative standard high pressure liquid chromatography (HPLC) and turbulent flow chromatography method chromatograms of 500 ng/kg (parts per trillion) tetracycline in fish matrix. This indicates the capability of removing endogenous interferences using the LC/MS system, thus reducing ion suppression effects and improving detection limits.

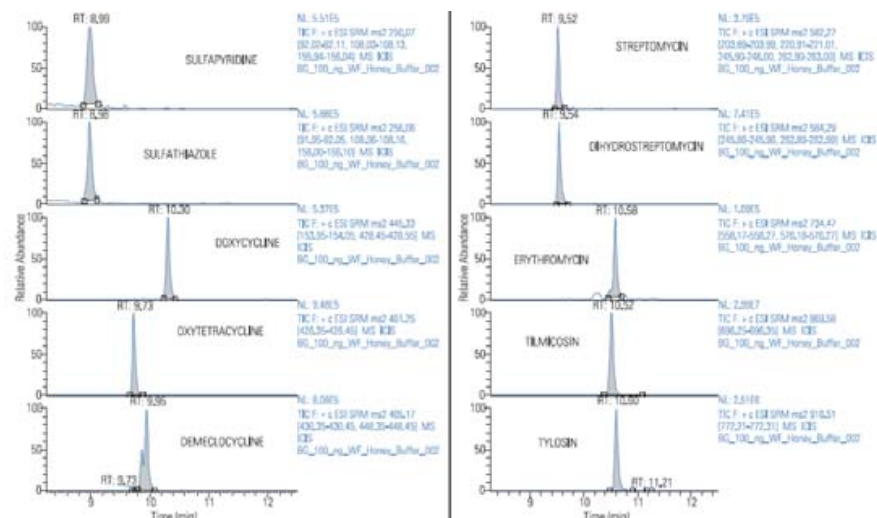
FIGURE 2. Chromatogram comparison of tetracycline at 500 ng/kg in fish (tilapia) matrix in standard HPLC and turbulent flow chromatography method



2) Antibiotics in honey

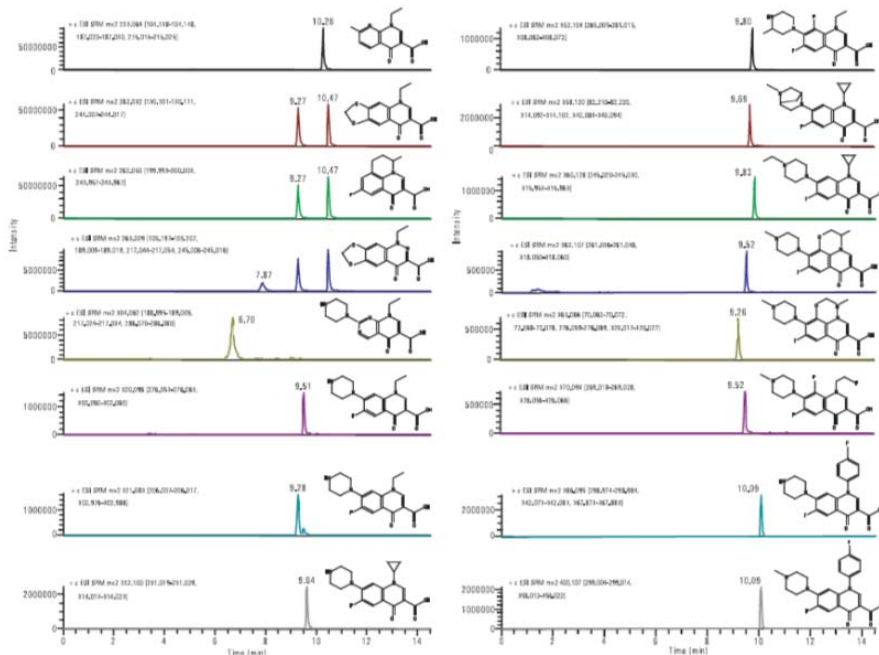
Ten representative antibiotics in honey belonging to four different structural classes were selected: sulfonamides, tetracyclines, aminoglycosides and macrolides[3]. The only offline sample preparation step required was the aqueous buffer dilution of raw honey to reduce the sample viscosity, which took less than 10 minutes. The total LC-MS/MS method run time was less than 18 minutes. A representative chromatogram of the 10 analytes at 100 ng/mL in 1:1 honey/buffer is shown in Figure 3.

Figure 3. Example chromatogram of 100 ng/mL calibration standard in 1:1 honey/buffer



Quinolones, including fluoroquinolones, in honey were also investigated[4]. Instead of using an SPE method, an online extraction method using turbulent flow chromatography was developed. The sample preparation time for the entire batch, including 16 compounds, was reduced from 5 hours to 40 minutes (80% of sample preparation time eliminated). The quantitation limits for the majority of analytes were 1 µg/kg (ppb) with no matrix interference. Figure 4 illustrates representative selected reaction monitoring (SRM) chromatograms at 20 µg/kg, showing the selected ion transitions and retention times for the studied analytes.

FIGURE 4. Figure 4: Representative SRM chromatograms (20 µg/kg) showing the selected ion transitions and retention times for the studied analytes



3) Pesticides in green tea

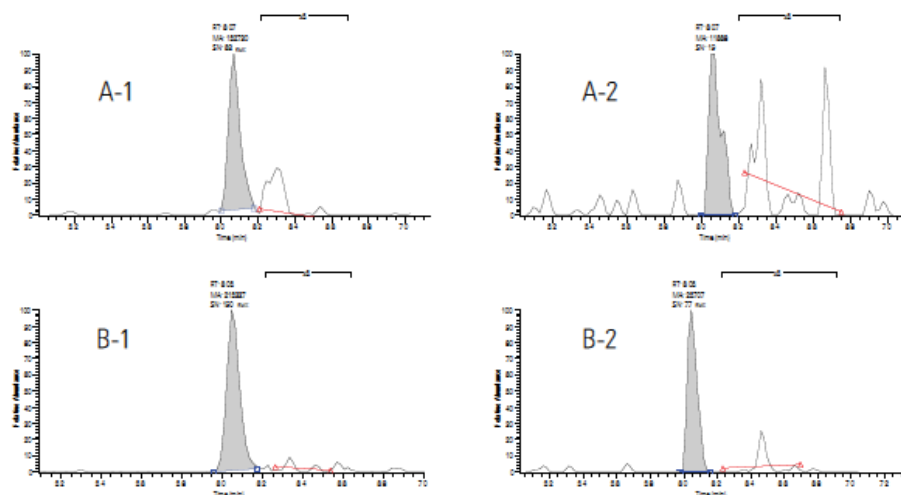
As shown in Figure 5, we compared a TurboFlow method and two currently popular methods for pesticide residue sample preparation, SPE and quick, easy, cheap, effective, rugged, and safe (QuEChERS). A typical SPE method involves equilibrating the cartridge, loading, washing and eluting analytes. It usually takes about 1 week to process 100 samples. Although QuEChERS was designed to simplify sample preparation, it still requires two-step centrifugation and concentration. A few days are typically required to prepare 100 samples with QuEChERS. TurboFlow technology minimizes preparation of 100 samples to less than 3 hours, dramatically improving the efficiency and throughput of this routine lab test[5].

FIGURE 5. Comparison of the TurboFlow method to SPE and QuEChERS

SPE	QuEChERS	TurboFlow Method
Weigh Sample	Weigh Sample	Weigh Sample
Extraction	Extraction	Extraction
SPE Loading	Shake and Centrifuge 5 min	Filtration
Washing	Transfer Top Layer to Clean-up Tube	LC-MS/MS
Eluting	Shake and Centrifuge 5 min	100 Samples, 3 Hours
Drying	Drying	
Reconstitution	Reconstitution	
Filtration	Filtration	
LC-MS/MS	LC-MS/MS	
100 Samples, 1 Week	100 Samples, 2 Days	

By using the Transcend TLX system with TurboFlow technology, the background noise and interference peaks are reduced significantly. Figure 6 compares chromatograms of Clomazone at 6.25 µg/L in tea extract using standard HPLC (top) and the TurboFlow method (bottom). The left panel (A-1 and B-1) shows the primary transition of m/z 240 > 125. The right panel (A-2 and B-2) shows the secondary transition of m/z 240 > 89. It clearly shows the effectiveness of background reduction using TurboFlow technology while the signal to noise ratio increased by 3 and 4 times for m/z 125 and 89 transitions, respectively. The area responses of both peaks also increase by more than 50% due to the minimization of ion suppression incurred by matrix. We also noticed the mass spectrometry response become more stable across the entire tested concentration range, thus improving the method reliability.

FIGURE 6. Comparison of chromatograms of Clomazone at 6.25 µg/L in tea extract using standard HPLC (top) and the TurboFlow method (bottom).



Conclusion

Online sample extraction utilizing turbulent flow chromatography coupled with LC-MS/MS and complimentary techniques has gained popularity in the food safety arena. The objective of this technology is to provide automated, high resolution, high sensitivity and high specificity separation of target analytes from extremely complex food matrices, removing the need for manual sample preparation and therefore increasing sample throughput. Turbulent flow chromatography also facilitates mass spectrometry detection and quantitative measurement and minimizes ion suppression and matrix effects. In addition, the multiplexing capability of certain LC/MS systems can quadruple the throughput of a turbulent flow chromatography method, providing unmatched productivity and cost savings.

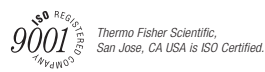
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