Safe and Authentic Foodstuffs

Waters ACQUITY UPC² System crucial to method development at CAIQ/FSI in China

TECHNOLOGY: ACQUITY UPC² SYSTEM

SCIENCE AND TECHNOLOGY AT CAIQ

The Chinese Academy of Inspection and Quarantine (CAIQ), a national public Institute, was established to research and develop science and technology for inspecting and quarantining foodstuffs and related commodities. CAIQ is a consolidation of two organizations, the Plant Quarantine Institute of Ministry of Agriculture (founded in 1954) and the China Import and Export Commodity Inspection Technology Institute (founded in 1979).

CAIQ consists of eight institutes and seven technical support centers. Within its organization CAIQ oversees 11 state key laboratories and employs about 1000 staff, including 400 researchers. CAIQ's research remit includes food safety, animal and plant quarantine, instrument analysis and health quarantine.

ASSURING FOOD QUALITY – THE FSI

The Institute of Food Safety (FSI) at CAIQ focuses on food safety research with a team of more than 40 scientists. FSI also manages the AQSIQ Key Laboratory of Food Safety and Import and export food inspection and quarantine reference laboratory. Research and method development for chemical residues, heavy metals and other toxic and hazardous substances are the focus of much of FSI's work. Its main mission is to carry out research projects for The People's Republic of China ministry of science and technology (MOST), and provide technical support to the General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China (AQSIQ), especially in food safety, import and export food safety and international cooperation.

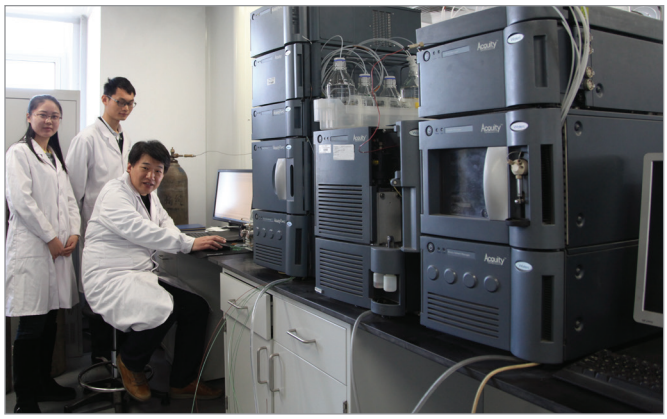
Professor Feng Zhang (front row, left) with staff and students in the CAIQ laboratory.

WORKING WITH WATERS

The Institute of Food Safety at CAIQ has enjoyed a working relationship with Waters since the company first established a direct office in China and currently has Waters LC, GC, LC-QTof-MS and ACQUITY UPC²® systems in the laboratory.

Professor Zhang commented: *"My first LC instrument as a postgraduate student in 1999 was a Waters Alliance® 2695 Separations Module, so when I became director of the FSI, one of my first decisions was to invest in the new UPC² system. The company has always provided us with excellent back-up. In addition to the high level applications and technical support we enjoy, I really appreciate the conferences and academic meetings that Waters organizes. These provide a place to network with other scientists and allow for good exchange of information. Waters is a very professional company to partner with."*

The ACQUITY UPC² system has proved straightforward to implement into the lab. *"My students receive around one week of basic training in SFC and the ACQUITY system, and then are able to start using it for their projects. Once someone is familiar with the principles, the system is very easy to use,"* elaborates Professor Zhang.



Professor Feng Zhang (front, right) with the ACQUITY UPC² system.

With substantial investment from both the "10th and 11th Five Year Plans", the FSI has been one of the leading proponents of food safety projects, and has won numerous awards including a first prize at CAIA (China Association for Instrumental Analysis) multiple times. The FSI also has a global collaboration program for food safety cooperation.

FSI's well-equipped laboratories include high-throughput systems, ultra-trace detection systems and a comprehensive range of separations and analytical instrumentation, notably LCMS and GCMS from vendors including Agilent, Thermo Fisher Scientific, Waters, AB Sciex, and Shimadzu. Waters instruments include:

- Waters GC-MS/MS
- Waters LC-MS/MS
- Waters ACQUITY UPC²
- Waters LC-QTof-MS

Professor Feng Zhang is the Director of the Institute of Food Safety, winner of the Mao Yi-Sheng science and technology prize, Commissioner of food quality and safety detecting instrument and technology application branch of China instrument and control society, and Honorary Professor of the College of food equipment engineering and science of XI'AN JIAOTONG University. Professor Zhang and his lab team is focused on solving technical problems in the process, and developing methodology, rather than being a regular inspection/analytical facility. There are around 20 employees and 15 graduate students, who work currently on between 10 and 20 projects per year. The Institute of Food Safety also works on a key special research project from "the 13th Five-Year Plan".

PUTTING THE AQUITY UPC² SYSTEM TO WORK

FSI has carried out several projects using the ACQUITY UPC² System including the separation and analysis of isomers in Chinese herbal medicine; the separation of heterocyclic amines whereby FSI's method reduced the analysis time from 22 (by GC-MS/MS) to 6 minutes (by UPC²); and also separation of isoflavones which took a 30-minute HPLC separation down to 7 minutes with the UPC² Technology.

In a recent project[1] FSI developed and optimized a novel method using ultra-high performance supercritical fluid chromatography combined with photo diode array detection (UHPSFC-PDA) to separate and identify 10 sulfonamides and five metabolites in serum.

There were several drivers for this work. As a large group of synthetic antibiotics, sulfonamides have good antibacterial effect on both gram-positive and gram-negative bacteria. In animal husbandry, sulfonamides are often used prophylactically, to avoid disease and promote growth. However, sulfonamide residues in meat or meat-derived products should not enter the food chain and many regulatory bodies have set strict limits for the presence of sulfonamides in food.

In order to understand the pharmacodynamics and pharmacokinetics of these compounds, a rapid, simple method for the detection of various sulfonamides and their metabolites in serum is required.

Up to now, methods have been based on sample extraction process, followed by HPLC. This is a complex analysis, that is not only time consuming and hard to automate, but also consumes significant amounts of organic solvents.

"Compared with traditional liquid chromatography, ACQUITY UPC² is very fast for sulfonamides analysis. LC and GC will not solve all the problems of food safety analysis perfectly."

PROFESSOR FENG ZHANG
Institute of Food Safety, Chinese Academy of Inspection and Quarantine (CAIQ)

For this project, the goal of the FSI scientists was to develop and optimize a simple, rapid method that was amenable to high-throughput processes when required, and that reduced the amount of solvent consumed per analysis. In addition, although the initial work was completed with serum samples, the method should also work with a range of other sample types.

The new method will be adopted by another department within CAIQ, which manages routine testing for import and export foodstuffs across China via its network of city-based laboratories. The method will also be adopted as the standard by other institutes throughout China.

Experimental

An ACQUITY UPC^2 system with a PDA Detector (set at 265 nm) from Waters analyzed 10 common sulfonamides along with the N-acetylated metabolites of five of these compounds. Initially, four different ACQUITY UPC^2 columns were investigated together with various mobile phase modifiers. The benchmark separation using the best combination of column and modifier was further improved by optimizing the back pressure, column temperature and flow rate. Serum samples were thawed and processed for analysis by a standard protein precipitation and centrifugation protocol.

Results

Having identified the best combination of column and modifier, the final optimized ACQUITY UPC^2 method conditions were: BEH column, ethanol as mobile phase modifier, backpressure 1800 psi, injection volume 2 µL, column temperature 35°C, samples maintained at 22°C, flow rate 1.6 mL/min.

Figure 1 shows the clear separation of sulfonamides achieved with this set-up. Furthermore, FSI found that this method compared favorably with the traditional HPLC, which separates just five sulfonamide drugs in a 10-minute protocol, whereas the ACQUITY UPC^2 system separated and quantified 10 different sulfonamides plus five important metabolites in around seven minutes.

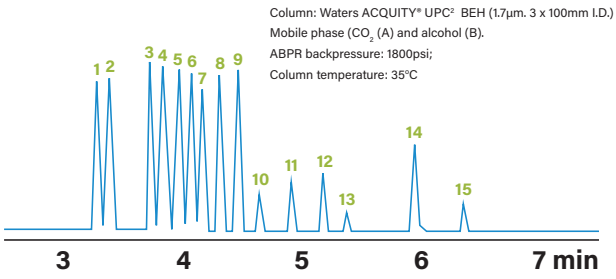


Figure 1. Optimized method conditions: BEH column, 95% A (initial), 95–70% A (0–8 min), 70–95% A (8–8.1 min), 95% B (8.1–9 min). A = CO_2, B = ethanol. Temperature 35 °C; ABPR 1800 psi; flow rate 1.6 mL/min.

Professor Zhang expects the new method will work with a range of sample types – this is important as food samples have long been considered one of the most difficult matrices to analyze.

ACQUITY UPC^2 TECHNOLOGY

Since its introduction in 2012, ACQUITY UPC^2 has enabled analytical chemists to reach far beyond conventional separations. The technology has been adopted successfully in laboratories around the world, where its characteristic robustness, speed, and reproducible performance are important.

Key characteristics of the technique:

- Considerably faster separations
- Ability to separate closely related compounds
- Capability to separate chiral compounds
- Significantly lower organic solvent use and waste management
- Streamlined processing due to much smaller dry down fractions as the main solvent – liquid carbon dioxide – reverts back to its gaseous state and vents away

What differentiates the ACQUITY UPC^2 Systems is Waters' patented sub-2-µm hybrid particle chemistry, which offers significant benefits over HPLC systems equipped with standard five µm particle chemistries.

The applications for the system used on its own, or paired with Waters optical and MS detection technologies, include: ADME screening, food safety, bioanalysis, clinical analysis, metabolite identification, and metabolomics, for example.

ADDING VALUE FOR THE FUTURE

In addition, new food adulterants will need to be measured, and existing compounds of interest will demand more detailed study. Professor Zhang explains: *"Currently people do not have to pay attention to the potential harmful effects of isomers, because with existing methods they appear to have similar toxicity. But as technology progresses, the evaluation of each isomer and its specific toxicity will be further refined, and I believe we will have a strong requirement to ensure the reliable separation and analysis of all isomers."*

He continues: *"We adopted the ACQUITY UPC² technology because of its improved robustness and performance. With ACQUITY UPC², FSI benefits from a green system that offers high throughput performance with time-saving benefits."*

"The refinements made to the UPC² System, specifically the pump and detector designed for supercritical fluids, has resulted in a separation tool that solves both routine and complex chromatographic problems, and is particularly useful for samples possessing a wide range of polarities."

CAIQ laboratory, Beijing.

Separation science is key to Professor Zhang's work, and he has extensive experience in liquid, gas, and supercritical fluid chromatography. *"LC and GC each have their own advantages, but I believe that SFC can be very helpful, providing us with faster, more environmentally friendly methods with increased separation capacity as well as more accurate qualitative and quantitative results,"* explains Professor Zhang.

FSI has applied the ACQUITY UPC² System to a wide range of separations challenges, and it seems likely that the potential of the system will grow even further.

"UPC² offers our scientists unique workflow, application, and environmental impact benefits compared to LC and GC platforms and will increasingly be deployed to develop methods for measuring pesticide residues, veterinary residues, toxins, illegal additives and nutritional ingredients," explains Professor Zhang.

Furthermore, the drive for increased sensitivity continues amid the move toward more stringent regulations around the world – from adding new substances to the list of restricted compounds, to lowering the permissible levels of those already regulated as we learn more about their metabolism and effects in the body – means that the analytical laboratory is constantly pressured to improve methods and embrace new technologies, such as ACQUITY UPC².

Professor Zhang added: *"It is also important to remember that we will, in the future, see much more non-targeted screening programs. In this situation, we face not only high demands on the analytical methods, but also the need to ensure that the downstream data processing, structural inference and identification protocols are all in place."*

References

1. Yuan Zhanga, Wei-E Zhoua, Shao-Hui Lia, Zhi-Qin Rena, Wei-Qing Lia, Yu Zhoub, Xue-Song Fengb, Wen-Jie Wua, Feng Zhang, 'A simple, accurate, time-saving, and green method for the determination of 15 sulfonamides and metabolites in serum samples by ultra-high performance supercritical fluid chromatography' Journal of Chromatography A: 1432 (2016) 132–139.

"The ongoing challenges of assuring the quality and non-adulteration of our food supply demand new analytical approaches. The ACQUITY UPC² gives us a fast and powerful additional tool that has already allowed us to create robust, rapid methods for a range of compounds of interest."

PROFESSOR FENG ZHANG
Institute of Food Safety, Chinese Academy of Inspection and Quarantine (CAIQ)

To find out more about applications for ACQUITY UPC²; or to download a primer on convergence chromatography, visit www.waters.com/upc2

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