

## Innovative Food Safety Method Advances Contaminant Detection in Aquatic-Based Food Samples

Waters Xevo TQ-S Triple Quadrupole Mass Spectrometer with APGC enables scientists to expand the detection and quantification of contaminants in food samples at the Research Institute for Pesticides and Water (IUPA), University Jaume I in Spain

### TECHNOLOGY: Waters Xevo TQ-S with APGC

#### FOOD SAFETY ANALYSIS AT IUPA

IUPA, located in Castelló de la Plana, Spain, comprises the Laboratory of Pesticide Residue Analysis (LARP), the Analytical Research Section and the Water Resources Group. In 2001 LARP obtained the Certificate of Fulfillment of Good Laboratory Practices for the analysis of pesticide residues (Certificate 17BPL/22), making it the first Spanish laboratory to achieve this certification in the field of pesticides. Since its creation, LARP has been directed by Professor Félix Hernández, who was also the first director of IUPA and supervisor of the Ph.D. of Tania Portolés.

Since then, IUPA has expanded into other fields of study – organic pollutants in the environment, aquaculture research, food safety, wastewater analysis, new psychoactive substances investigations, and metabolomics in clinical and food quality/authenticity fields, among others.

Most of IUPA's work uses coupled techniques based on chromatography (gas and liquid) and mass spectrometry using quadrupole, ion trap, triple quadrupole, time-of-flight (ToF), and quadrupole time-of-flight (QTof) instruments. The organization has 10 laboratories with approximately 30 researchers.

Researchers at IUPA at University Jaume I (UJI) began working in 2011 to explore an innovative technique for the detection and quantification of contaminants in food samples, including of marine origin.

The European Union has limited or prohibited the use of many of these compounds, but their established presence in the food chain makes monitoring these contaminants in food essential to ensure that the levels do not exceed the allowable concentration.



Dr. Tania Portolés in the Laboratory of Pesticide Residue Analysis, IUPA, UJI, Spain.

#### WORKING WITH WATERS

Having worked together previously, Waters® asked Dr. Portolés to test the Waters Xevo® TQ-S in combination with APGC – a request that came at a fortuitous time. Her team was already working to overcome the limitations of EI ionization for food safety analysis, and the collaboration with Waters proved to be the answer they were looking for.

Dr. Portolés explains:

*"Serendipitously, Waters came to us and invited us to test a prototype of the APGC instrument. Working with Waters is exceptional. They try to help you with any difficulty you may experience with their instruments, via e-mail or in situ. We're always learning, even after six years. Their maintenance programs are quite useful, as they take care of any instrument issues and try to solve the problems within a few days. We've always had a good relationship with Waters."*



The team at IUPA, UJI, Spain.

## URGENT NEED FOR FOOD CONTAMINANT MONITORING

Brominated flame retardants (BFRs) are harmful and toxic compounds present in food – mainly seafood, since they are introduced into the oceans through waste, residues or discharges from the factories that produce them.

Monitoring the presence of these contaminants is vitally important for food safety, particularly because they are used extensively by manufacturers to reduce flammability of common products such as televisions, smart phones, furniture, and plastic products in general.

Dr. Tania Portolés explains: *“These pollutants reach the marine environment and are ingested by fish and shellfish. Since they are lipophilic substances, they cannot be excreted – they remain in the fats. Thus, we consume them when we eat a piece of tuna, for example. At high levels, they are toxic and can cause health problems including carcinogenesis, endocrine disruption and neurological problems.”*

IUPA researchers wanted to develop advanced analytical methodology to improve the monitoring of these compounds in food and environmental samples. Using atmospheric pressure gas chromatography (APGC), they found an innovative method that is very effective for identifying and quantifying these contaminants.

## IMPROVED SENSITIVITY AND SELECTIVITY

Traditionally, the determination of BFRs and other persistent organic pollutants used electron ionization (EI) as an ionization technique.



Dr. Tania Portolés, IUPA, University Jaume I.

However, this technique can lead to extensive fragmentation and the specific molecular ion is either not present or has a low intensity. This lack of specificity makes the identification of these compounds difficult and can also reduce the sensitivity.



**“APGC is a more sensitive and selective technique because if we have the whole molecule we can detect with greater confidence if there are contaminants in the sample and in what proportion.”**

DR. TANIA PORTOLÉS  
IUPA, University Jaume I

Recent advancements in technology, however, are overcoming these problems. IUPA utilized a new chemical ionization source, atmospheric pressure gas chromatography (APGC), developed by Waters, that results in a “soft” ionization process.

Dr. Portolés began exploring the use of APGC as a new technique for detecting contaminants in aquatic-based food sources with the Waters Xevo TQ-S Mass Spectrometer. In previous work, she found the increased sensitivity of the Xevo TQ-S instrument enabled IUPA scientists to quantify and confirm trace components at lower levels in the most complex samples.

Furthermore, the IUPA team found the analysis of food samples by APGC allows for improved selectivity when generating multiple reaction monitoring (MRM) transitions in comparison with the significant fragmentation experienced with EI source. Operating the gas chromatography system at atmospheric pressure provides increased scope for ionization modes – namely charge and proton transfer.

## PUTTING THE TECHNOLOGY TO THE TEST

Dr. Portolés used APGC to develop and test a method that would increase the number of contaminants detected, at much lower concentrations than previous methods reported, in a variety of food samples.

Collaborating with the Institute of Aquaculture Torre la Sal in Spain and the National Institute of Nutrition and Seafood Research (NIFES) in Norway, IUPA researchers used the Waters Xevo TQ-S with APGC for GC-MS/MS analysis of PAHs, polychlorinated biphenyls (PCBs) and pesticides in 19 different matrices – including fish tissues, feeds, and feed ingredients<sup>1</sup>.

## BACKGROUND AND CHALLENGE

The method used gas chromatography coupled to triple quadrupole tandem mass spectrometry with an atmospheric pressure chemical ionization source (GC-APCI-MS/MS). The method is based on a modification of the unbuffered QuEChERS method, using freezing as an additional clean-up step and applying a 20-fold dilution factor to the final extract.

In addition to the 24 PAHs, researchers tested for 15 pesticides and 7 PCB congeners to widen the scope of the method. The study was to determine trace levels (as low as 0.1 ng/L) of PAHs, PCBs, PBDEs, and some emerging flame retardants.

## Experimental

The team used a total of 76 samples from 19 different matrices. The list contains ingredients from different origins (plant, terrestrial animals, and marine), and feeds based on these ingredients (PAPs not included), as well as fillets of Atlantic salmon and gilthead sea bream reared on these feeds.

Fish individuals were fed for 7 and 18 months, respectively, and fillet samples were taken for analysis at the end of the exposure trial and additionally for sea bream at 8 months (commercial size). The same feed compositions were provided throughout the feeding trial.

The quantification was performed on feed ingredients, feeds produced from the same feed ingredients, and fish fillets of fish fed on these feeds. The feed samples were analyzed at the beginning of the trial (additionally after 8 months for sea bream) and no stability assessment was made by analyzing the feed during storage (below 7 °C for salmon and sea bream feeds).

## Outcome

The high sensitivity of this technique allowed the simultaneous quantification of 19 different complex matrices from aquaculture using solvent calibration. The excellent sensitivity and selectivity provided by GC-APGC-MS/MS allowed the dilution of the sample extracts and quantification using calibration with standards in solvent for all the 19 matrices tested.

One of the study's key contributions was the elimination of matrix effect, thereby removing the need for time-consuming purification steps. The developed method was evaluated at 2, 5, and 50 ng g<sup>-1</sup> spiking levels. LOQs of the developed method were 2 ng g<sup>-1</sup> for most analytes with LODs in the range of 0.5 to 2 ng g<sup>-1</sup>. The results also showed increased selectivity in the determination and identification of contaminants.

Analysis of real-world samples revealed the presence of naphthalene, fluorene, phenanthrene, fluoranthene and pyrene at concentration levels ranging from 4.8 to 187 ng g<sup>-1</sup>.

No PCBs, DDTs or pesticides were found in fillets from salmon and sea bream.

## IMPROVED SENSITIVITY FOR PESTICIDE DETECTION

The IUPA study found APGC in combination with the Waters Xevo TQ-S instrument is a robust and sensitive technique for analyzing a broad range of contaminants in several marine-based matrices.

As a result, the study concluded:

- The possibility of selecting the molecular ion or the protonated molecule as a precursor ion for MRM experiments provides greater sensitivity and selectivity.
- This excellent sensitivity and selectivity allows for the dilution of the sample extracts and quantification using calibration with standards in solvent, so matrix matched calibrations can be avoided in some cases.
- When using an APGC source, gas flow rates higher than 2 mL/min can be used without loss in ionization performance, allowing an improvement in detection response, enhancing resolution of critical pairs. Therefore, significant speed improvement can be achieved.
- The method is capable of promoting M<sup>+</sup> or [M+H]<sup>+</sup> formation with the addition of modifiers. Therefore, it allows for modifying the ionization efficiency according to the compounds and the aim of the experiment.

**Improved ability to measure complex matrices:** The increased sensitivity of the Xevo TQ-S Mass Spectrometer with APGC enabled IUPA scientists to quantify and confirm trace components at even lower levels in the most complex samples.

One of the key findings was the ability to eliminate the matrix effect, thereby eliminating the need for time-consuming purification steps. That's a huge time savings for IUPA researchers.



**"If you couldn't do it by dilution and you had to do it by sample matrix calibrations, it would be a lot of work."**

DR. TANIA PORTOLÉS  
IUPA, University Jaume I

Furthermore, the APGC technique reduces the cost of tests for contaminants because it is more sensitive and selective. It would use less solvents and materials in comparison with the previously used techniques and would be able to determine the compounds at a lower concentration.

The Waters Xevo TQ-S instrument is equipped with unique StepWave™ technology, enabling detection limits at ultra-trace levels.

This allows compliance with regulatory limits and the ability to inject less sample matrix, reducing effects of contamination on the GC-MS system and therefore increasing uptime.

**Soft ionization:** The “soft” ionization of APGC was a game changer for the IUPA team because of the reduced fragmentation for many compounds when compared with techniques such as EI. Reduced fragmentation can give higher sensitivity and specificity, therefore simplifying pre-cursor ion selection in MS/MS analyses.

Dr. Portolés elaborates: *“The improvement was very important. Using the soft ionization approach with the latest generation of triple quad instrumentation added a lot of sensitivity and specificity to our work.”*

That’s a big advantage for IUPA scientists, who need to analyze a broad range of contaminants in variety of matrices.

Dr. Portolés adds: *“APGC is an attractive alternative to EI (less fragmentation) and CI (more universal) and opens new and promising perspectives in quantitative analysis at trace levels and also in universal wide-scope screening. With this interface, a soft and reproducible ionization is favored in GC, being the protonated molecule and/or the molecular ion the base peak of the spectrum in most cases. This notably facilitated the application of MS/MS methods (with triple quad or Q-ToF), and also the screening of contaminants with GC-MS focusing the search to the highly diagnostic molecular ion.”*

**Easy LC to GC changeover:** Another big advantage of the Waters Xevo TQ-S instrument is the quick and simple changeover from LC to GC with one APGC method. Since APGC is not a vacuum technique equilibration, time between techniques is kept to a minimum. This means the analysis can be tailored to demands, maximizing up-time and instrument utilization. It also gives researchers the widest possible coverage from analyses.

Dr. Portolés explains: *“The versatility of the technique is high because you can have both GC and LC coupled to the same mass spectrometer. It is quite quick and easy to change from LC to GC and vice versa. The system is not difficult to use, and it is quite user-friendly after proper training.”*

The ease of changing from LC to GC is so unique to the Waters Xevo TQ-S that often IUPA’s collaborators don’t believe it’s possible until they see it for themselves.

Dr. Portolés adds: *“They cannot imagine how you can change in five minutes. So we need to show our colleagues how it works.”*

IUPA’s research using APGC has brought more opportunities to collaborate because it’s attractive to other researchers or organizations interested in exploring the use of APGC for their own studies or expanding its use to new compounds. That is important for an academic research institution.

Dr. Portolés explains: *“Companies that work with us are interested in knowing the capabilities of APGC in their project. In all cases, we try to collaborate with some other research group because the work you can do is more interesting. You get different point of views, and the research is richer.”*

The implementation of this innovative technique has great implications on food security, both to monitor food lots with a very high concentration of pollutants, in which case they would be recalled, and to monitor that they meet the standards regulated by the European Union.



*“Right now if we have any project with GC-amenable compounds and we want to do quantification of those compounds, we always use APGC because it’s the best we have. If we need sensitivity, it’s the instrument of choice.”*

DR. TANIA PORTOLÉS

IUPA, University Jaume I

## References

1. T. Portolés, B. Garlito, Jaime Nácher-Mestre, M.H.G. Berntssen, J. Pérez-Sánchez, ‘Multi-class Determination of Undesirables in Aquaculture Samples by Gas Chromatography/Tandem Mass Spectrometry with Atmospheric Pressure Chemical Ionization: Novel Approach for Polycyclic Aromatic Hydrocarbons’, *Talanta*, 172 (2017) 109–119.
2. Tania Portolés Nicolau; J.G.J Mol; Juan Vicente Sancho Llopis; Félix Javier Hernández Hernández. Advantages of Atmospheric Pressure Chemical Ionization in Gas Chromatography Tandem Mass Spectrometry: Pyrethroid Insecticides as a Case Study. *Analytical Chemistry*. 22 (84), pp. 9802–9810. 2012.
3. Tania Portolés Nicolau; Carlos Sales; Belén Gómara; Juan Vicente Sancho Llopis; Joaquín Beltrán Arandes; Laura Herrero; M<sup>a</sup> José González; Félix Javier Hernández Hernández. Novel Analytical Approach for Brominated Flame Retardants Based on the Use of Gas Chromatography-Atmospheric Pressure Chemical Ionization-Tandem Mass Spectrometry with Emphasis in Highly Brominated Co. *Analytical Chemistry*. 19 (87), pp. 9892–9899. 2015.

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