# Fully Automated QuEChERS Clean up and LC/MS-QQQ Analysis of Pesticides in Fruits and Vegetables

***This article outlines a fully automated method based upon the widely used QuEChERS (quick, easy, cheap, effective, rugged and safe) methodology -, for the extraction and clean up of pesticide residues in samples of fruit and vegetables. With the number these measurements set to rise in the near future, the commercial case for the automation of this time consuming, manual method is compelling.***

***The QuEChERS method uses an acetonitrile extraction followed by the salting out of water from the sample using anhydrous magnesium sulphate, sodium chloride and buffering citrate salts to induce liquid-liquid partitioning. This initial extraction is performed offline and provides a crude extract ready for QuEChERS clean up.***

***An automated dispersive solid phase extraction (DSPE) is conducted for clean up, using a combination of primary secondary amine sorbent (PSA) to remove fatty acids and anhydrous magnesium sulphate to reduce the remaining water in the extract. After mixing and centrifugation the upper layer is ready for analysis.***

> ***"Samples of fruit and vegetables were bought from a supermarket and prepared in accordance with standard protocols; (chopped into small pieces, frozen and blended) to give homogenous samples."***

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The determination of pesticides in fruits and vegetables is required to ensure food safety. Sample preparation is an important part of any multi-residue method. Automating the sample clean-up and coupling the robot directly to Liquid Chromatography Mass Spectrometry – Triple Quadrupole (LC/MS-QQQ) holds the promise of dramatic improvements in laboratory productivity by streamlining the analytical process and presents opportunities to improve data quality at the same time

The methodology and hardware may be used in two different configurations, either as a stand-alone Robot performing the DSPE clean up in isolation, (i.e. not directly coupled to LC/MS-QQQ), or alternatively in an special configuration, coupled directly to LC/MS-QQQ, providing the greatest levels of laboratory productivity available *(Figure 1).*

*Figure 1. Anatune automated LC-QQQ system configured for online QuEChERS.*

## METHODOLOGY

Samples of fruit and vegetables were bought from a supermarket and prepared in accordance with standard protocols; (chopped into small pieces, frozen and blended) to give homogenous samples.

Portions of these samples (10g) were weighed into extraction tubes in preparation for EN QuEChERS extraction. Samples of apple, strawberry, lettuce, tomato, spinach and oranges were stored, frozen until required.

For each matrix, samples (n=3) were spiked at 10ng/g, which represents a level appropriate to the maximum residue limits (MRLs) for the matrices and compounds under investigation. Additional samples were processed un-spiked to provide enough extract for the preparation of matrix matched calibration standards and blank analysis.

For the offline extraction, 10g of sample were extracted with 10ml of acetonitrile. Internal standard (triphenyl phosphate (TPP) 50ng/g) was added and the sample was shaken for one minute. Next the EN mixture of salts was added to buffer and remove water from the samples. The sample was then thoroughly shaken for one minute to mix the salts with the fruit/solvent slurry. This mixture was then centrifuged to separate the solids from the crude acetonitrile supernatant/extract, which was then ready for automated DSPE clean up.

For the automated DSPE a 1ml aliquot of this crude extract was added to a vial containing 25mg of PSA sorbent and 150mg of magnesium sulphate ($MgSO_4$). This mixture is then vortexed to ensure a high degree of mixing and to ensure maximum interaction between PSA and $MgSO_4$ and the crude extract. The mixture was then centrifuged at 3,000rpm for three minutes. For the samples of lettuce and spinach, graphitised carbon black (GCB) was also present in the clean-up vial to ensure sufficient removal of pigments and chlorophyll.

The clean supernatant was filtered using the robot's SPE Station to 0.45µm through PTFE filters, to ensure that the extract was suitable for rapid resolution LC/MS-QQQ.

In the case of online QuEChERS automation, the resulting filtrate was injected into an LC/MS-QQQ in a 'just in time' fashion ensuring that each analysis was always completed as soon as extracts become available.

From the 200 pesticides analysed, 24 were selected for data comparison purposes. This selection was made based upon the availability of recently published recovery data, for manual clean-up using QuEChERS.

Linear calibrations for all compounds, in each matrix were achieved in all cases. (*Figure 2*) Matrix blanks were also analysed during the analysis to provide evidence for any analyte contribution arising from the sample itself; all instrument blanks for all matrices for the compounds under investigation showed no positive determinations *(Figure 2).*

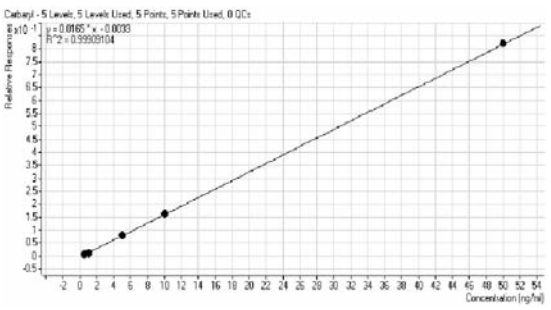


*Figure 2. Carbaryl calibration curve 0.5 – 50 ng/g.*

The use of TPP as an internal standard at 50ng/g allowed the generation of calibration curves by plotting the relative responses of analytes (peak area of analytes/peak area of TPP) against the relative concentration of the analyte (concentration of analyte/concentration of TPP). Successful determination of the 1ng/g calibration standard in all matrices showed that the quantification limits (LOQ) were lower than the required MRLs.

The recovery and reproducibility of this method was evaluated by spiking the matrices with an appropriate amount of multi-component standard to give a concentration of 10ng/g.

These samples were quantified against the matrix matched calibration of 0.5 to 50ng/g to ensure any suppression or enhancement effects due to the presence of matrix impurities was catered for. The analysis was performed in triplicate (n=3), the data for the selected 24 pesticides, show that acceptable recoveries (74 to 120%) are generated with an average of 100%. The % RSD ranged from 0.2 to 19.3 with an average of 6.5, which is acceptable for quantitative analysis at a spike of 10ng/g.

## RESULTS

Chromatograms from the analysis showed sharp, well resolved peaks as can be seen in *Figure 3*.

The recovery and reproducibility data based upon the matrices studied at the levels spiked show that the system is fully suitable for automating the QuEChERS DSPE clean up. Automating this process ensures that the entire procedure is fast, easy, and offers time and labour savings, while ensuring consistency. The hardware can process 50 samples in roughly 11 hours.

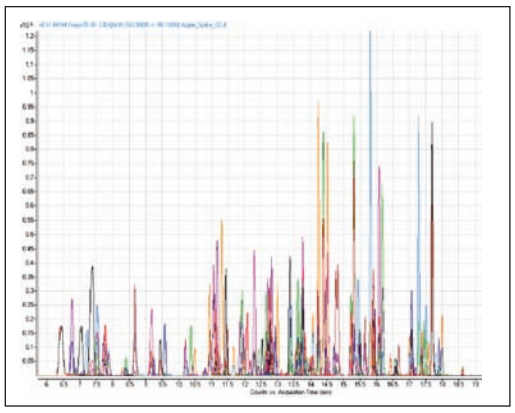

*Figure 3. Chromatograms from the analysis of spiked apple samples showed sharp well resolved peaks.*

## CONCLUSIONS

This system offers a viable alternative to the manual process. Samples may be cleaned-up and analysed in a 'real-time' fashion and this ensures that each sample is treated in exactly the same way and that analytes are exposed to the absorbents for the minimum time, minimising opportunities for sample degradation and improving reproducibility. The system has a capacity of 98 samples, permitting laboratories to gear-up for increasing work-loads. The high degree of automation ensures that the incremental cost of performing more analyses is kept to a minimum.

Full details of this work are contained in Anatune chromatography technical note: AS90 Fully automated QuEChERS clean-up and LC/MS-QQQ analysis of pesticides in fruits and vegetables, Copies of this are available from the author.

## ACKNOWLEDGEMENT

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# Mass Spectrometry Technology Helps Children's Hospital

**Thermo Fisher Scientific, Inc** announced that a team of researchers at Children's Hospital Boston identified a promising biomarker, known as LRG, that they believe will help physicians diagnose acute pediatric appendicitis more accurately and efficiently. The team conducted its research on a Thermo Scientific LTQ Orbitrap mass spectrometer and discovered several potential biomarkers, including leucine-rich alpha-2-glycoprotein (LRG). Biomarkers are biological indicators, commonly detected in blood, urine or tissue samples, which can reveal key risk indicators for a disease, the presence of a disease or shed light on how a disease may develop in an individual. In addition to their use in pre-clinical research and clinical diagnosis, biomarkers play a significant role in the early treatment of certain diseases.

The research was inspired by the reality that appendicitis diagnosis often requires long hours in an emergency room and expensive diagnostic imaging procedures. Even worse, incorrect diagnosis leads to unnecessary surgeries – in one out of every ten to twenty appendectomies a healthy appendix is removed. The Children's Hospital team, led by Hanno Steen, PhD, director of the Proteomics Centre at Children's Hospital Boston, with Alex Kentsis, MD, PhD and Richard Bachur, MD of the Division of Emergency Medicine, used an LTQ Orbitrap™ mass spectrometer to study over an 18-month period protein and peptides in urine samples from 67 children. Dr. Steen's findings show that the LRG biomarker is a consistent and accurate indicator of appendicitis and that LRG levels directly correlate with the severity of infection.

"Given the time spent diagnosing appendicitis, the radiation exposure due to computed tomography, and the number of unnecessary surgeries, we're naturally very excited about the implications of identifying these biomarkers," said Dr Hanno Steen. "The next step for us is to look for biomarkers for adult appendicitis and fulfill our vision of developing a rapid urine test, using a simple dipstick, to accelerate testing and improve accuracy and reliability. The Children's Hospital Boston team is demonstrating the promise of mass spectrometry for breakthrough medical research by using it to discover biomarkers that will ultimately impact patient care," said Robert Kane, Sales Representative at Thermo Fisher Scientific. "In the past few years, this technology has advanced – with greater speed and precision – to a point where scientists are discovering biomarkers more rapidly."

Circle no. 57

# Rapid and Convenient Substance Extractions Directly into MS


The new **Camag** TLC-MS Interface is a versatile instrument for extracting compounds from a TLC/HPTLC plate and feeding them into a mass spectrometer for substance identification or structure elucidation. Surveys have shown that not all samples can be processed by HPLC-MS or HPLC-DAD systems alone - due to no or low detection of the compounds or impurities in the UV range, a heavy matrix load, or a lack of MS compatibility with the solvents that are necessary for the HPLC separation. In such cases the addition of TLC/HPTLC to the system may be the ideal solution. In the past unknown substances were scraped off the TLC/HPTLC plate, eluted into a tube and transferred into the MS. Now a convenient and universal TLC-MS Interface is available which can semi-automatically extract zones of interest and direct them online into a HPLC-MS system.

The device can be connected to any brand of LC-coupled mass spectrometer. The semi-automatic TLC-MS Interface is quickly and easily connected (by two fittings) to any LC-coupled MS without adjustments or mass spectrometer modifications. In lieu of plate scraping Substances of interest are extracted directly from a TLC/HPTLC then pumped into the MS, producing within a minute sensitive MS signals per substance zone. The interface extracts the complete substance zone with its depth profile, allowing detections comparable to HPLC down to the pg/zone range.

Circle no. 58

# Calibration Software Enables Single Quadrupole Instruments to Easily Identify Unknowns

**Cerno Bioscience** and **Agilent Technologies, Inc** announced they will jointly market Cerno's MassWorks calibration software to Agilent GC/MSD ChemStation users. This novel calibration increases the mass accuracy of Agilent's single quadrupole GC/MSD sufficiently to identify unknown compounds. This normally requires use of much more expensive, larger instruments such as Quadrupole Time-of-Flight, or Fourier Transform Ion Resonance mass spectrometers.

MassWorks for GC/MSD uses Cerno's patented technology to calibrate raw MS data from Agilent's GC/MSD using peak shape, followed by CLIP formula ID based on spectral accuracy. This technique dramatically improves mass accuracy and enables single-quad instruments to identify unknown compounds. In addition to enabling compound identification without a library, the formula ID function also speeds and adds confidence to library searches. The combined capabilities of the Agilent GC/MSD Chemstation and Cerno's MassWorks means there is now an economic alternative to high-resolution GC/MS systems for formula ID.

"The combination of Agilent's GC/MS with Cerno's MassWorks holds real value for end users," said Chris Toney, Agilent Vice President and General Manager, Mass Spec Systems. "Customers can use their workhorse single-quad instruments to identify unknowns rather than rely on more specialised instruments with associated costs and delays."

"We're delighted to enter into this relationship with Agilent, a recognised market leader in GC/MS. With Agilent's large install base and worldwide reach, we will be able to make MassWorks available to many more users," said Dr Yongdong Wang, Founder and President of Cerno Bioscience. "By configuring and pricing MassWorks specifically for the GC/MS market, we can make this solution more attractive for all GC/MS laboratories."

MassWorks is compatible with Agilent 5975 and 5973 GC/MS instruments running Agilent MSD ChemStation software. To determine compatibility with older systems, please contact Cerno or Agilent.

Circle no. 59