Pesticide analysis EZ

When the sample matrix no longer matters

Fully automated Sample clean-up and Pesticide Screening with Agilent 6410 LC/MS QQQ online SPE System, Agilent ordering Number: 5990-3866EN

Application specialists from TeLA GmbH have developed a new method that dramatically simplifies LC/MS determination of pesticide levels, providing high-quality results independent of the sample matrix type and complexity.

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Pesticides, fungicides and herbicides are needed in order to provide an adequate supply of food to the ever-growing human population across the world. The other side of the coin is that residues of these types of compounds in foods cannot be allowed to endanger or affect the health of the consumer. To ensure that foods do not endanger us, maximum acceptable levels, sometimes referred to as tolerated levels, have been established for individual compounds according to the current state of scientific knowledge. If these levels are exceeded, it would be illegal to market the contaminated product in Europe. Corresponding laws were established by the EU. This legal basis must be, or, in some cases, already has been, adapted into National law by EU member states.

In Germany, the details on maximum acceptable levels of residues can be found in the German LFGB, acronym for the compendium of laws governing Food, Feed and various consumer products. As an aside, the term "consumer products" in this context spans a great variety of products ranging from packaging that comes into contact with food, feed or personal care products to personal care products themselves, such as cosmetics, tooth paste or shampoo or other personal care items that make more than brief contact with skin or mucous membranes.

Rules, unless properly enforced, are of course worth less than the proverbial paper they were printed on. In other words trust is fine, but we should verify and if needed take corrective action to ensure compliance and best possible consumer safety. This requires a network of reliable laboratories, which is not a trivial matter, as can be seen a bit later in this text.

World-wide, around 700 pesticides are in use, very few of which can be legally used throughout Europe. Various compounds classes have been established, but even these can cover a wide range of polarities, making it difficult to develop a fast all encompassing analysis method.

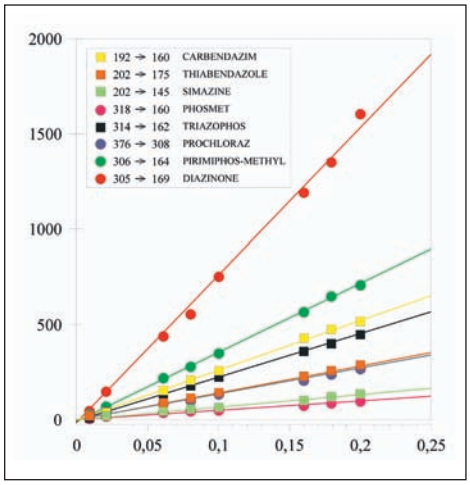
Still, effective multi-residue methods are in use for the determination of pesticides, helping to ensure food safety. When fruits and vegetables are analyzed for pesticide residues, often several pesticides are found. The effects on human health have only been documented for very few of these compounds or compound groups.

Tracking down pesticides using GC/MS and LC/MS

Classical pesticide analysis relied on gas chromatography (GC) using an electron capture detector (ECD) or a nitrogen phosphorous detector (NPD). The most widely used detector today is the mass selective detector (MSD).

In Germany, the analytes that are mainly in focus are those listed in the DFG S19 method, a multi-residue method for the determination of pesticides in food, which enjoys Europe-wide recognition. The analysis of the 270 compounds listed in the S19 method does, however, require significant sample preparation including a gel chromatography clean-up step to separate analytes from the matrix.

Different analysis techniques are used for different types of pesticides. Liquid chromatography (LC) combined with a mass selective detector (MS) is used to determine polar to moderately apolar compounds. Gas chromatography (GC), most often in combination with a mass selective detector (MSD) covers apolar to moderately polar compounds. As can be seen from this description, there is some overlap between the techniques. Recently a new multi-residue method for the determination of pesticide levels in fruits and vegetables was presented (QuEChERS: Quick, Easy, Cheap, Effective, Rugged & Safe) [*]. Compared to previous methods, the QuEChERS sample preparation steps are much less time-consuming, enabling the preparation of 8 samples in less than 30 minutes. QuEChERS is a sample preparation method well suited for both GC, GC/MS and LC/MS analysis. The QuEChERS sample preparation steps are listed below.



Calibration curves for nine pesticides, determined using the TeLA GmbH SPE-LC-MS/MS pesticide multi-residue method.

The main benefit of this sample preparation method is that the overall analysis is less time-consuming and less error-prone than more traditional approaches. Unfortunately, extracts obtained following this procedure often have a high matrix content, which causes chromatographic problems for GC analysis due to residue build-up in the liner unless an automated liner exchange system such as the GERSTEL ALEX is used. (Cf.: GERSTEL Solutions Worldwide Magazine No. 5 p. 18) (http://www.gerstel.com/solutions_no5.htm)

QuEChERS method:

Weigh 10 g of sample
–> Add 10 ml of Acetonitrile (AcN)
Shake vigorously 1 min
–> Add 4 g MgSO4 and 1 gNaCl
Shake vigorously 1 min
–> Add internal standard solution
Shake 30 sec and centrifuge
–> Take Aliquot of supernatant
–> Add MgSO4 and sorbent
Shake 30 sec and centrifuge
–> Take Aliquot of supernatant
–> inject to GC-MS and LC-MS

The results obtained using QuEChERS sample preparation are comparable to those reached using the S19 method. The QuEChERS method is much faster, requires much less sample preparation, covers a wider range of analytes and is more readily automated. In addition, much smaller volumes of partly toxic organic solvents are required, compared with other currently used methods for determining pesticides in fruits and vegetables. In addition to the financial benefits of a much higher laboratory throughput, the cost of materials at around one Euro per sample is relatively low.

The limits of QuEChERS are encountered whenever samples with more complex matrices need to be analyzed, such as garlic, onion, artichoke or avocado

[*] M. Anastassiades, S. Lehotay, D. Stajnbaher and F. Schenck: Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. J AOAC Int 86 (2) (2003) 412-31.

Norbert Helle and Meike Baden in front of the SPE LC-MS/MS system used for the pesticide multi-residue method: Agilent Series LC 1200 and a GERSTEL SPE system mounted across an Agilent 6410 MS/MS Triple Quad.

with much higher fat content. This can lead to problems with interferences, than can especially influence quantification unless further clean-up steps are performed.

To enable reliable and rugged analysis independent of the sample matrix, we looked for a similarly effective alternative sample preparation procedure. We found that automated solid phase extraction (SPE) based on the GERSTEL MultiPurpose Sampler (MPS) provided an excellent solution. The GERSTEL SPE, we have previously used successfully for a number of applications, including aflatoxins, chloramphenicol and malachite green in foods. In summary, we can report that our automated SPE-LC-MS/MS-ESI multi-residue method reduces the number of manual steps required to a minimum while increasing laboratory throughput. The results are solid and reproducible combined with high sensitivity and good limits of determination.

Instrumental requirements

The GERSTEL SPE was fitted with an injection valve; sample introduction to the Agilent LC 1200 was performed directly by the SPE system; detection was performed using an Agilent 6410 MS/MS Triple Quad instrument.

Sample Preparation: 15 mL of an acetonitrile/water mixture (80:20) was added to a five gram sample of fruit or vegetable for extraction. The SPE cartridge (M&N C-18ec, 6 mL, 1 g) was conditioned using 10 mL methanol (MeOH) and 10 mL water. All steps in the sample preparation procedure, including sample introduction were fully automated.

5 mL sample was added to the cartridge, which was subsequently rinsed with 5 mL water. Analytes were then eluted using an acetonitrile/water mixture added at a flow rate of 600µL/min. In contrast to most manual SPE methods, the liquid is not aspirated through the cartridge under vacuum, rather it is added under positive pressure using a syringe. This means that flows, and therefore also the elution speed, are accurately controlled and results more reproducible. This holds true even when sample matrix changes the restriction across the cartridge. The eluate was concentrated for six minutes at 50 °C and the residual analytes taken up in 5 mL of a acetonitrile/formic acid mixture (30:70).

Sample introduction and analyte separation: 20 µL of the cleaned-up extract was introduced directly to the LC/MS-MS System. The temperature of the column (ZorbaxXDB C-18 100x2.1 mm, 1.8 µm rapid resolution) was set to 50 °C; flow rate: 0.5 mL/min resulting in a column head pressure of approximately 420 bar. A solvent mixture of 5mM formic acid (A) and acetonitrile (B) was used as mobile phase based on the following gradient programming: 0 min (20 % B); 5 min (20 % B), 30 min (90 % B).

Detection: Analytes were detected with positive Electron Spray Ionization (ESI) using the electron spray ion source or, alternatively, the Agilent Multimode ion source. Our experiments clearly showed that the Multimode source provided significantly lower detection limits for some pesticides than the ESI source. For other compounds, however, a lower response was obtained than with the ESI ion source. The settings for the ion source were optimized for the flow and eluent used. The following parameters were used: N_2 temperature: 340 °C; carrier gas flow (N_2): 9 L/min; nebulizer pressure: 30 psi. The triple quadrupole instrument was operated in MRM mode, with 5 different time segments, monitoring two transitions for each pesticide. In each segment 40 to 50 analytes were monitored.

The proof of the pudding

When using the QuEChERS method, it is necessary to adapt the clean-up steps to the sample at hand. It has been clearly shown that for "uncomplicated" matrices, such as lettuce or cucumber, additional clean-up steps are not required following the acetonitrile/water extraction.

For complex matrices that contain fat and other challenging matrix components, further clean-up steps are of course needed. For this purpose we used the GERSTEL SPE system.

Raw sample extracts were automatically loaded onto standard SPE cartridges and cleaned. A new cartridge was used for every sample to eliminate cross-contamination. Macherey-Nagel cartridges containing C18 reversed phase material were found to produce excellent, reliable results.

Automated SPE clean-up as described in this article took around 20 minutes to complete. Apart from the first sample, the SPE process was performed dur

ing LC/MS or GC/MS analysis of the preceding sample, ensuring that the SPE step was performed without increasing the overall analysis time. Once the first sample had been prepared for analysis, the LC/MS or GC/MS system never had to wait idly for the next sample.

An LC 1200 Rapid Resolution HPLC system from Agilent Technologies was used for the analysis. In order to achieve good separation combined with method ruggedness, the conscious decision was made to only seek a moderate reduction of the analysis time. The total analysis time required to determine around 140 compounds was in the order of 35 minutes. This time period was more than sufficient to prepare the following sample for just-in-time sample introduction to the LC/MS system.

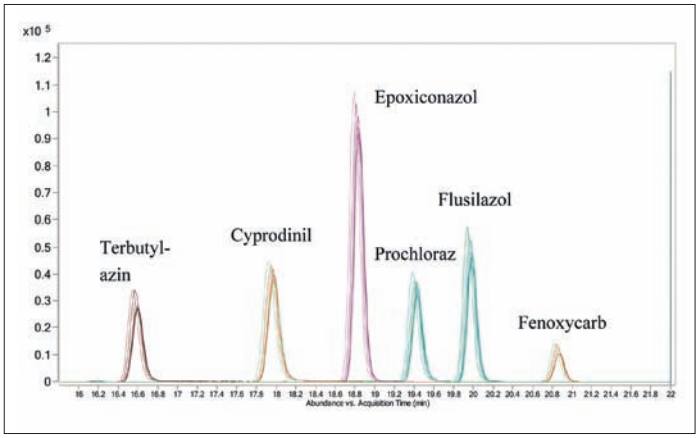
Sample clean-up using SPE contributes not only to the ruggedness of the method, it also improves reproducibility and linearity, among other things. To illustrate this, a bell pepper sample was spiked with a pesticide mixture and analyzed.

Following SPE clean-up, retention times and peak areas of the analytes showed excellent reproducibility. The linearity was excellent, both for polar compounds like Carbendazim und Thiabendazole as well as for apolar pesticides like Diazinon und Pirimiphosmethyl.

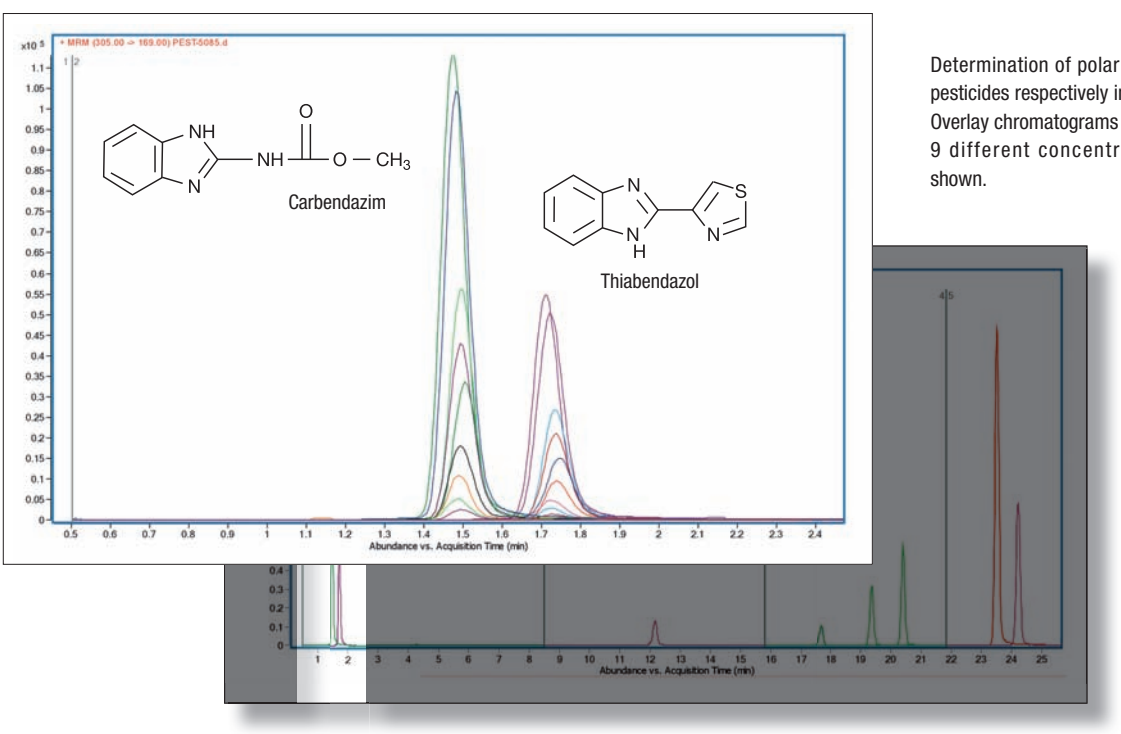
Orange oil samples were cleaned up using a slightly modified SPE method. The efficiency of SPE clean-up is illustrated by the fact that the intense yellow color of the sample was transferred to the cartridge while the resulting extract was a clear and colorless liquid. Recovery for the various compounds in this difficult matrix ranged from 70 to 90 % while recoveries from fruit and vegetable samples were mainly in the range from 80 to 100 %. It is worth noting that the Zorbax SB-C18 Rapid Resolution columns used provided excellent peak symmetry.

One final comment: Every method must prove its worth in practice. The test, as always, is in the analysis of real world samples. To prove the validity of our method, we took part in a Europe-wide round robin with 46 participating laboratories. A vegetable sample (zucchini) had to be analyzed for 185 different pesticide residues. Out of 46 laboratories, TeLA GmbH was among the 12 that managed to correctly identify and quantify the analytes thus meeting the round robin requirements and passing the test.

128 of the 185 pesticides were determined using our SPE-LC-MS/MS pesticide multi-residue method. 90 of the 185 pesticides were determined using a GC/MS system (GC 6890 / MSD 5973, both from Agilent Technologies) in combination with the GERSTEL MultiPurpose Sampler (MPS) using a Retention Time Locking (RTL) method.



Overlay medium polarity sections of 8 different chromatograms: 8 separate sample preparations and injections of a bell pepper sample spiked with a standard mixture of pesticides, 100 ng/mL each. The peaks shown are for the pesticides Terbutylazin, Cyprodinil, Prochloraz, Flusilazol and Fenoxycarb, all showing good reproducibility.



Determination of polar and apolar pesticides respectively in orange oil. Overlay chromatograms covering 9 different concentrations are shown.