A Q&A

Extraction and Measurement of GC-amenable Pesticides in Difficult Matrices

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The routine quantitation and identification of pesticide residues at low concentrations are among the most important and demanding applications in food safety. Typical workflows, use a combination of liquid and gas chromatography techniques, combined with mass spectrometry, for the comprehensive analysis of hundreds of different pesticides in hundreds of different sample matrices. Small-scale generic solvent extraction methods based on the QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) approach have gained in popularity and have proved to be widely applicable. Nonetheless, some issues have been reported when using QuEChERS for the extraction of pesticides from tuber crops like yam, taro, and sweet potato. Fortunately, the use of an alternative method based on the Accelerated Solvent Extraction technique with elevated temperature and pressure, has enabled fast and efficient solvent extraction of pesticides from tubers. Analysis of these extracts, using the Thermo Scientific™ TSQ™ 8000 Evo Triple Quadrupole GC–MS/MS system, enables fast, selective and sensitive detection, and quantification, of pesticide residues in these challenging matrices. To find out more about the use of the accelerated solvent extraction technique and GC-MS/MS, LCGC spoke with Kaushik Banerjee and Zareen Khan at the ICAR-National Research Centre for Grapes, National Referral Laboratory in Pune, India.

Michael Swartz: Your laboratory is a National Referral Laboratory for pesticides in India. Can you describe the main functions of the laboratory and its role in the pesticides community?

Kaushik Banerjee: The National Research Centre for Grapes belongs to the Indian Council of Agricultural Research. It is a Ministry of Agriculture research institute and received National Referral Laboratory recognition in 2004. We organize proficiency tests, assist testing labs throughout India, and approve laboratories to perform regulatory and commercial testing. We also organize capacity building programs for labs and other stakeholders in the agro-industry. Futhermore, we conduct risk assessment studies, carry-out various field and laboratory-based experiments on pesticides and other agrochemicals, and facilitate development of agricultural practice-based recommendations for farmers and various stakeholders in the agriculture industry.

Michael Swartz: India is such a diverse country in terms of climate and agriculture. You must see a kaleidoscope of different food products. Can you briefly describe your main pesticides workflows to deal with this diversity and identify the sample types which are the most troublesome?

Kaushik Banerjee: We have developed many generic sample extraction procedures for the analysis of food contaminants in a range of matrices including fruits, vegetables, and various processed commodities, like wine and dried fruits. Analyses are performed predominantly by GC-MS/MS and LC-MS/MS on triple quadrupole platforms. Our laboratory performs regular

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research to address the many matrix effects encountered during the analysis of vegetables like okra or green chili, and crops such as taro, yam, and spices. These commodities are commonly encountered in India, and are quite complicated in nature. Validated methods for most of these matrices are not available in any literature, so we modify and optimize sample preparation methodologies for these matrices then transfer them to training and capacity building programs, as well as testing laboratories across the country. The SANTE 11945/2015 guideline is the QC procedure used for method validation and in our laboratory training program.

Michael Swartz: Can you briefly describe the main issues encountered with these difficult matrices and measures taken to overcome the challenges?

Kaushik Banerjee: The main issues we deal with while optimizing analytical procedures for complicated matrices, include poor and inconsistent recovery, and unacceptable matrix effects. To overcome these issues, we improve the sample preparation procedure by utilizing the accelerated solvent extraction technique in combination with the selectivity and sensitivity of GC-MS/MS and LC-MS/MS instruments.

Michael Swartz: When using the Accelerated Solvent Extraction technique, which parameters need to be optimized to be able to obtain valid results compliant with the regulations?

Zareen Khan: There are three main parameters to consider while developing an effective accelerated solvent extraction method; temperature, static cycle time and number of static cycles. Temperature is set and maintained above the boiling point of the extraction solvent, with the help of high pressure. Under these conditions the solvent penetrates the sample matrix thoroughly, providing more efficient extraction of the analytes compared to use of solvents at room temperature. Simultaneously, we also have to take care to prevent or minimize degradation of the target compounds. When developing a new method, we recommend a temperature range of 75°C to 125°C, with 100°C as the default. The static cycle time is the time the sample and solvent remain in contact with each other at a high temperature and pressure during extraction. A minimum extraction static time is used. Increasing the static time unnecessarily will lengthen the total extraction time and may result in undesired matrix components in the final extract. A static time cycle is defined by each time fresh solvent is introduced into the extraction cells. Traditionally, one or two static cycles have been used for most applications. While additional static cycles will compensate for the lack of fresh solvents during the heated step, the resulting extracts will be more diluted, requiring a proportionate re-concentration. We found that three static cycles of three minutes each, in place of one 10-minute static step, provided optimum analyte recovery and laboratory efficiency. Partitioning the final extract with water helped to achieve a visibly clear extract and a subsequent decrease in matrix effects for around 20% of the pesticides. This in-turn results in higher recoveries of target compounds compared to using other extraction methods.

Michael Swartz: Has the accelerated solvent extraction method made a difference to your laboratory?

Zareen Khan: Yes. It has automated the sample preparation workflow, reducing the number of manual steps during pre- and post-extraction, while also reducing the exposure of the analyst to organic solvents. The total duration of the entire extraction is similar in ASE compared to manual extraction procedures like QuEChERS. Since the accelerated solvent extraction method allows unattended extraction of 24 sequential samples at a time, it increases the sample output and lab productivity by more than 60%.

Michael Swartz: Do you find that the acquisition of several MS/MS transitions for each analyte possible with modern GC-MS/MS systems is useful when analyzing complex matrices?

Zareen Khan: Yes, the TSQ 8000 Evo Triple Quadrupole GC-MS/MS system, allows acquisition of several selected reaction monitoring (SRM) transitions for each target molecule, which is useful since sometimes selectivity can be matrix-dependent. In such cases, a particular transition could be associated with a false positive signal from the matrix. The transition providing the highest selectivity and abundance is selected for quantification, whereas the other interference-free MS/MS transitions are selected for identification and confirmation.

Michael Swartz: Can you provide a brief overall summary of the recovery precision and quantification limits obtained using your workflow that combines the accelerated solvent extraction method and GC-MS/MS?

Zareen Khan: For ASE extraction of 160 compounds of different chemical classes, that were analyzed by GC Evo Triple Quadrupole MS, most recoveries are between 70% and 120%, with precision RSD less than 20%, and with LOQs less than 10 ppb.

Michael Swartz: You've shown excellent results for GC-amenable pesticide residues. Do you have any data for LC-amenable pesticides?

Zareen Khan: Yes, we have conducted similar studies in combination with Triple Quadrupole LC-MS/MS, and we have found satisfactory results for over 300 target LC-amenable pesticides of various chemical classes. Recoveries were found to be between 70% and 120%, with precision RSD less than 20%, and with LOQs less than 10 ppb.