Results and Discussion

An MRM method was developed targeting 108 transitions representing 18 pesticides in order to aid in a reliable screening and quantitation protocol using Agilent Ultrafast QuEChERS kits (5982-1560) and UHPLC/MS/MS methods established in the company's laboratories. This method enables rapid analysis of matrix effect interference, sample dilution which allows reduction in matrix effects, speed of sample analysis, and significantly better recoveries were observed after dilution.

Methodology

Our approach was to use optimized MRM transitions for 5 mycotoxins and 5 dyes. All but best MRM transitions via optimization of inter-MRM delay times, and the compound recovery data were also taken from the Agilent Agilent Small Molecule Library for MS/MS. The instrumental conditions for this method were performed on an UHPLC/MS/MS system comprising an Agilent 1290 Infinity II LC with an Agilent 6495 Triple Quadrupole Mass Spectrometer, and an Agilent MassHunter Qualitative Analysis software to perform both the MRM analysis.

Method Development and Performance

An MRM method was first developed with new transitions optimized for mycotoxins and dyes compounds of interest. Next, the new compound transitions and obtained RT data were used to update an MRM method targeting >250 pesticides.

The combination of UPLC with an optimized Triple Quadrupole Mass Spectrometer allowed the quantitation of the majority of analytes at an LOQ of 2 ppb or lower as required for the regulatory documentation. However, for certain mycotoxins, dyes, and pesticides, the lower limit of quantitation was <1 ppb. Excellent assay precision (RSD %) <12% at LOQ and <5% at the rest of the levels were shown in the table below. Correlation coefficients (R²) for calibration curves were higher than 0.99 over up to 4.7 orders of linear dynamic range.

Figure 2. Example of the dynamic range and linearity achieved using Agilent tMRM method. The driving force behind this performance is the excellent sensitivity of the 6495 LC/MS system coupled to high-performance liquid chromatography (HPLC) and high-performance sample cleanup.

Conclusions

Optimized intermediate MRM delay times allowed the use of UPLC dwell times as short as 0.5 ms with minimum intensity reduction. Mycotoxin and dye compounds were incorporated into a UPLC/MS/MS pesticide method for the determination of more than 250 compounds.

Black pepper was identified as the most challenging spice matrix we analyzed. As shown in Figure 3, a 10% dilution of the blank extract was required to achieve acceptable sensitivity. However, the matrix effect data demonstrated the sensitivity of the 6495 LC/MS system enabled analysis of the desired 111 black pepper dilution level while maintaining the ability to detect the majority of the compounds with an IDL of 10 pg.

Spices are very challenging food matrices, due to their complex nature results in significant ionization suppression effects. Matrix effects were further enhanced in our study as the sample volume and sample matrix effects and improved method robustness.

Figure 5. Example of the dynamic range and linearity achieved using Agilent tMRM method. The driving force behind this performance is the excellent sensitivity of the 6495 LC/MS system coupled to high-performance liquid chromatography (HPLC) and high-performance sample cleanup.

Minimizing Matrix Effects by Dilution of Extracts

The ability to obtain high sensitivity with acceptable matrix effects enables more accurate quantification, while also reducing chromatogram data quality by lower matrix effects, thereby reducing robustness. The beneficial effect of dilution is shown in the table below, whereas compounds recoveries in black pepper improve as the sample is further diluted.

Table 1. Recoveries for selected compounds calculated for different dilution levels of a black pepper sample solution in a quality control sample (mean ± RSD) (n=3).