How to Minimize Matrix Interferences in GC/MS/MS Analyses?

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Introduction

Food extracts after sample cleanup are usually still very complex containing various matrix residues such as high-boiling compounds. These matrix components can cause contamination and deterioration of GC sample path and MS ion source. Different matrix cleanup protocols have been studied but could not eliminate matrix residues injected into a GC/MS system. Matrix can cause quantitation interference, lower response (higher LOQ), and/or bad peak shape resulting in poor data quality.

Besides using column backflush and new ultra-inert inlet liners with wool, this presentation demonstrates an easy approach to work around matrix interferences to ensure high data quality.

Experimental

A representative group of 33 challenging pesticides were selected for trace level analysis in six different plantation food matrices - white flour, strawberry, pear, orange, pepper, and spinach, using QuEChERS sample preparation protocol [1].

Solutions and Standards

A 20 µg/mL 33-pesticide mixture was made in Acetone by dilution of individual pesticide stock solutions, and stored at 4 °C. In order to minimize matrix dilution in the calibration standards, a 500 ng/mL intermediate spiking solution was freshly made in the corresponding matrix blank from the 20 µg/mL standard mixture. The intermediate spiking solution in matrix was then used to spike five **matrix-matched** calibration standards of 1, 5, 10, 50, and 100 ng/mL and a 10 ng/mL QC standard.

Experimental

Internal Standard

Internal standard (IS) stock solution of triphenyl phosphate (TPP) at 2 mg/mL was made in Acetone. A 20 µg/mL IS spiking solution in acetonitrile was made from the IS stock solution, and stored at 4 °C. Proper volume of IS spiking solution was then added into all samples to generate a concentration of 100 ng/mL.

Matrix Blank Preparation

The extraction procedure was described in detail in Agilent Application Notes [2,3]. The fruits and vegetables were frozen, chopped, and then homogenized thoroughly. In brief, 15 g of homogenous sample (except flour sample) was extracted using 15 mL of acetonitrile with 1% acetic acid and separated into aqueous phase by the addition of BondElut QuEChERS AOAC extraction salt packet (p/n 5982-5755). For flour sample, 5 g of homogenous sample was mixed with 10 mL of water and soaked overnight. This mixture was then extracted following the QuEChERS procedure. After centrifugation, the supernatant was transferred and cleaned up using the general dispersive SPE kit (p/n 5982-5022). After vortex and centrifuge, the supernatant was transferred into vials as matrix blank for subsequent experiments. These individual matrix blanks were stored at 4 °C.

Instrumentation

All analyses were done on an Agilent 7890 GC equipped with an Agilent 7693B autosampler and an Agilent 7000 series GC/MS Triple Quadrupole system in MRM (Multiple Reaction Monitoring) mode. Up to seven transitions for each analyte were used in the acquisition method. An Agilent Ultra Inert GC column, HP-5MS UI, was used to provide analyte separation and a highly inert flow path into the detector.

Results and Discussion

Chemical Background from the Matrix

A typical MRM database or acquisition method has two MRM transitions for each analyte. Figure 1 shows extracted ion chromatograms (EICs) of the top two transitions of methamidophos (at 10 pg) in two matrices. The retention time of methamidophos is about 4.6 minutes. The transitions are arranged in the descending order of responses with the larger one on top. Figure 1 shows the obvious issues of getting inaccurate quantitation results due to medium or strong matrix interference. For orange matrix, an overlapping peak in the second transition marked by a blue arrow, affected integration results and the qualifier ion ratio. For pear matrix, an overlapping peak in the first transition, marked by a green arrow, which is typically used for quantitation, gave higher and inaccurate quantitation results.

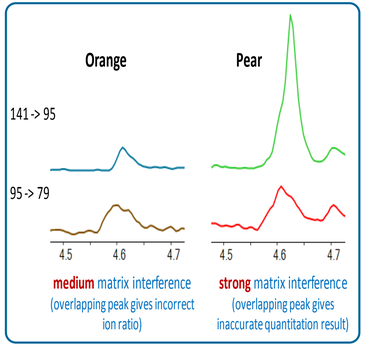

Figure 1. Top two transitions of Methamidophos (at 10 pg) in two matrices.

If a user only has two MRM transitions available for each analyte, it is difficult to work around the matrix effect as seen in Figure 1. The G9250AA database [4] has in average of **eight** transitions for each compound. This allows the user to choose alternative transitions easily when matrix interference affects peak shape and integration results.

In this study, seven transitions for each analyte were used in the MRM acquisition method.

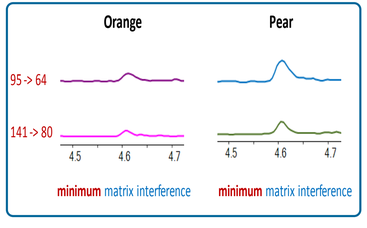

Figure 2. Two alternative Methamidophos (at 10 pg) transitions with minimum matrix interference

Figure 2 shows EICs of two alternative methamidophos transitions in the database. Both transitions showed minimum matrix interferences in orange and pear matrices. In fact, the EICs of these two transitions showed minimum matrix interference in all matrices in the study. Although these two transitions do not provide the highest responses, they are the optimal transitions for a universal or screening MRM method. It is always best to evaluate the chemical background of an analyte's multiple transitions in different matrices before selecting the most appropriate transitions in a particular matrix.

Results and Discussion

Signal-to-Noise Ratios

Evaluating the signal-to-noise ratios (S/N) of MRM transitions is another way to identify matrix effects. Some pesticides showed consistent MRM responses in different matrices, but many pesticides had different MRM responses in different matrices due to either matrix enhancement or matrix suppression.

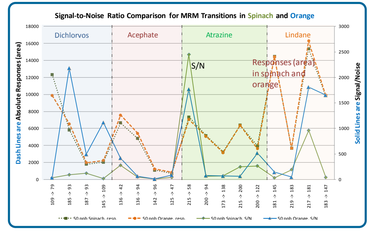

Figure 3. Signal to Noise ration comparison in spinach and orange

Figure above shows responses (i.e., area counts) and S/N's of several MRM transitions for four analytes in spinach and orange matrices. The orange dashed line and dark green dotted line represent area counts from MRM transitions. These dashed/dotted lines superposed tightly. The solid blue and green lines represent the S/N from the same MRM transitions. The solid lines showed significant variations for some transitions. This figure shows that the chemical noise of a particular transition can vary significantly in different matrices.

Using atrazine as an example, the area count for transition 215 -> 58 was about the same (approximately 7,000) from both matrices, but the S/N for this transition in spinach (~14,500) was about 40% higher than the S/N in orange matrix (~10,500). In contrast, for transition 200 -> 122, the S/N in orange matrix (~3,000) was about double the S/N in spinach (~1,500), even though the area counts from both matrices were about the same (approximately 4,000).

This matrix effect was not unique to atrazine. The S/N variations from some of the MRM transitions of dichlorvos and lindane were more pronounced even though the area counts were comparable in both matrices. Again, if the number of MRM transitions available for each analyte is limited to two or three, it is difficult to select optimal MRM transitions suited for the matrix analyzed.

The multiple transitions available in the G9250AA MRM database allow users to choose several optimized transitions to achieve accurate confirmation and quantitation results. This study showed that MRM transitions should be chosen according to matrix to achieve optimal and reliable quantitation results. It is important to use matrix-matched calibrations and low background transitions to achieve accurate quantitation results.

References

1. M. Anastassiades and S.J. Lehotay, J. AOAC Int., 2003, 86, 412- 431.
2. L. Zhao, D. Schultz, and J. Stevens, 5990-4068EN.
3. L. Zhao and J. Stevens, 5990-4305EN
4. C.-K. Meng, 5990-9453EN

Conclusions

Matrix can cause quantitation interference, lower responses, or poor peak shape. Each matrix has a different matrix effect. Therefore, it is critical to choose the most selective transitions for a particular matrix and use matrix-matched calibration curves to achieve accurate and reliable quantitation results. The G9250AA MRM Database has in average of eight MRM transitions with relative intensities for each compound to provide alternative measurements to minimize matrix interference. Easy-to-use tools as well as tutorial videos are also included in the database to build a full MRM acquisition method based on your list of compound CAS numbers in less than 10 minutes.