ROOT CAUSE DETERMINATION OF MATRIX EFFECTS IN LC/HRMS ESI

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Overview

The matrix effects in liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS) analysis affect the accuracy of results, are difficult to compensate for and present a major challenge in environmental and food safety analysis. An understanding on the root cause of the matrix effects allows for the implementation of viable approaches during the method development stage to resolve matrix effects in routine LC-MS analysis. Using calibration standards of 381 pesticides prepared in solvent and five different sample matrices as model samples, studies were performed to evaluate the relationship between analyte concentrations and sample matrices using high performance and micro-flow liquid chromatography-ESI-high resolution mass spectrometry (HRMS).

Introduction

The development of electrospray ionization (ESI) interface based liquid chromatography-mass spectrometry (LC-MS) instrumentation has gained tremendous momentum since the turn of the century. Using MS of different technological platforms, LC-MS analytical methods have been developed for the analysis of polar organic contaminants such as pesticides, antibiotics, pharmaceuticals, personal care products , cyanotoxins and mycotoxins in complex environmental and food matrices. These LC-MS methods are characterized by their superior performance in sensitivity, selectivity and the ability to use rugged sample preparation procedures without requiring extensive cleanup or the derivatization step that is commonly required by gas chromatography-MS based analytical methods. The ability of LC-MS to analyze samples prepared with less cleanup and no derivatization requirements also allowed for the development of multi-residue methods capable of analyzing >>600 pesticides in one analysis. The LC-MS based analytical methods suffer from matrix effects that are common in environmental and food analyses and that impact the data quality and accuracy of quantitative analyses. Matrix effects are commonly observed as ESI suppression (ESIS) of analyte intensity and have been studied by many researchers since the middle of the 1990s. Recognized by Kebarle and Tang in 1993 with detailed mechanisms explained by Bruins, ESIS occurs when the total number of molecules (i.e., effective concentration of analyte and matrix component) exceeds the prescribed number of molecules that an ESI source can handle. The surface area of ion emitting droplets was estimated (and may vary depending on the specific ESI source) to have a capacity accommodating about 6.02x1018 charged ions/L (or about 1x10-5 M) maximum. As long as the total concentration of analyte and matrix components is 1x10-5 M or less, the ESI source can effectively ionize all analytes eluted from the LC and no matrix effects of analyte(s) will be observed.

Methods

A Thermo Scientific™ Dionex™ UltiMate™ 3000 HPLC system consisted of a HRG-3400RS binary pump, WPS-3000 autosampler, and a TCC-3400 column compartment was used in the HPLC analysis. A Thermo Scientific Dionex NCS-3500RS system was used to carry out MFLC analysis (Figure 1).

FIGURE 1. Q Exactive High Resolution

Separations of the 1x, 10x and 100x diluted samples (108 total) were achieved on a Restek Ultra Aqueous™ C-18 (uAqueous, 3 □m, Bellefonte, PA, USA) and a Thermo Scientific Accucore aQ™ C-18 (Accucore, 2.6 □m shell with a 1.9 □m inner core, San Jose, CA) HPLC columns (100 x 2.1 mm with 10 x 2.1 mm guard cartridges). Separation for the 1000x diluted samples (30 total) was achieved on a Thermo Scientific Acclaim™ C18 (Acclaim, 3 □m, San Jose, CA) column (150x1 mm). Injection volumes of 15 and 5µL were used, respectively, in the HPLC and MFLC analysis. Mobile phases used were A: 5 mM HCOONH4, 0.1% HCOOH in CH3OH:H2O = 5:95 v/v and B: 5 mM HCOONH4, 0.1% HCOOH in CH3OH:H2O = 95:5 v/v. The HPLC separation used an initial equilibration time of 3 min at 5% B. Gradient separation was achieved from 5% B at 0 min, increased to 20% B at 4 min, 40% B at 5.5 min, 98% B at 10.5 min and maintained at 98% B until the end of the analysis at 15 min. The MFLC analysis used the same gradient elution except for the organic 98% phase which ended at 12.5 min. Column temperature was 35°C. Injection volumes were 15 and 5 µL, respectively for the 2.1 mm and 1 mm HPLC columns. Flow rates were 350 µL/min (uAqueous) and 420 µL/min (Accucore) for the 2.1 mm HPLC column and 30 µL/min (Acclaim) for the 1 mm i.d. MFLC column to achieve column back pressure ranges from about 3000 to 3500 PSI.

Sample and Standard Preparation

All sample matrices (avocado, orange, spinach, honey and hazelnut) were cryo-ground by blending broken pieces of a half-size block of dry ice in a Blixer 4 blender (Robot Coupe USA Inc., Jackson, MS) until a powdery consistency was obtained. Sample matrices were prepared by weighing 10 ± 0.2g of cryo-ground sample in 50 mL disposable screw capped polypropylene centrifuge bottles (Fisher Scientific, Pittsburgh, PA). These sample tubes were vortexed for 1 min to achieve a homogeneous sample followed by the addition of 10 mL CH_3CN, 4 g anhydrous $MgSO_4$, and 1 g anhydrous sodium chloride (UCT, Bristol, PA). After hand-shaking the sample to prevent clumping of the salts, the sample tubes were placed on a GenoGrinder® mechanical shaker (SPEX Sample Prep, LLC, Metuchen, NJ) for 1 min at 1000 strokes/min. Samples were then centrifuged at 4500 rpm x 5 min to separate the sample and extracts. The final extracts (~ 9 mL) were transferred through 0.2 µ nylon membrane filters (Sun SRi, Rockwood, TN) directly into 15 mL glass centrifuge tubes and used in the matrix effect study. The matrix materials in these solutions were estimated to be 1.1 g/mL.

Six intermediate calibration standards of 381 pesticides were prepared in CH_3CN at six concentration levels of 400, 200, 100, 50, 10 and 5 ng/mL (ppb). Using a calibrated electronic Eppendorf pipette, 100 µL of these six intermediate calibration standards were quantitatively transferred into six separate, 1.5 mL autosampler vials and treated as a sample set. Six of these six sample sets were used for the preparation of one SOCS and five MMCS 1x dilution samples at concentration levels of 40, 20, 10, 5, 1 and 0.5 ppb. This was done by adding exactly 900 µL of CH_3CN or sample extracts into each vial using a second, calibrated electronic Eppendorf pipette. Solutions in each of these 36 vials were homogenized for 30 sec on a vortex at 100 RPM. The 10x diluted samples were prepared by diluting 100 µL of these 1x samples using 900 µL of CH_3CN; resulting in concentrations of 0.05 to 4 ppb. The 100x diluted samples were prepared using 100 µL of 10x homogenized samples and 900 µL of CH3CN resulting in concentrations of 0.005 to 0.4 ppb. The 1000x diluted samples were prepared in the same manner by two consecutive serial dilutions of five, 1x concentration level standards from 20 to 1000 ppb; resulting concentrations from 0.02–1 ppb for the 1000x diluted samples. The 1x, 10x and 100x diluted samples would have 36 samples/level (six concentration levels for one SOCS and five MMCS sample sets) for a total of 108 samples while the 1000x level would have 30 samples used in the analysis. The experiment was designed to have those 1000x diluted samples analyzed by micro flow LC (MFLC) and to have analyte concentrations overlap the 10x and 100x and matrix materials diluted by 1000x. After removing 100 µL of solution from each of the 100x and 1000x diluted SOCS and MMCS samples, 20 µL of internal standard 2H10-diazinon was added into all sample vials to compensate for possible sample volume variation, homogenized on a vortex at 100 RPM and stored in freezer at −5° C until ready for analysis.

Experimental Design

The signal of the analyte in a solvent only solution SOCS (duplicate) is compared to that of a 1-1000 X diluted post-extraction spiked sample MMCS (duplicate) at the same concentration (matrix-matched standard). Any significant difference in response indicates (Figure 2.) either ion suppression or enhancement.

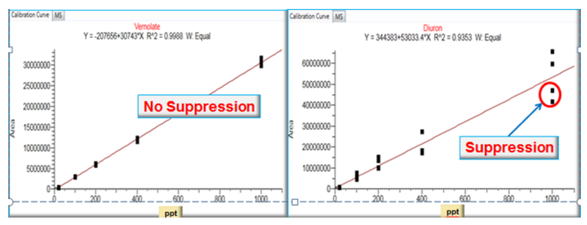

FIGURE 2. Comparison on ion suppression vs. N0 ion suppression

Results and Discussion

Figure 3, (a) XIC of Anilofos at 315.17050 (M+H)+, the left side was analyzed by UHPLC and the right side was performed by MFLC with the same 1ppb concentration by different dilution (10X and 1000X) factor on Avocado matrix. It is clearly demonstrated that the ion intensity of MFLC (5.25E6) has 10 times better sensitivity compare to the UHPLC analysis (5.06E5) on XIC plot at the same concentration of 1 ppb (ug/L). The calibration curves (d) were consisted by two sets of spiked samples in each calibration curve, which including solvent spike and matrix spike (Avocado) of the concentration from 0.02 ppb to 1 ppb to investigate the matrix interference or the degree of ion suppression in each calibration plot. It is shown that the curve was analyzed by the MFLC had more severe matrix interference even with 1/1000 dilution on same avocado extract compares with 10 times of dilution on the spiked sample at the left side of this Figure 3 (d). The reason for the severe ion suppression by the MFLC analysis is due to the ratio of the coeluted matrix ions (301.25317 + 319.26379) compare to the target analyte of Anilofos (368.05256) is more than 340 times.

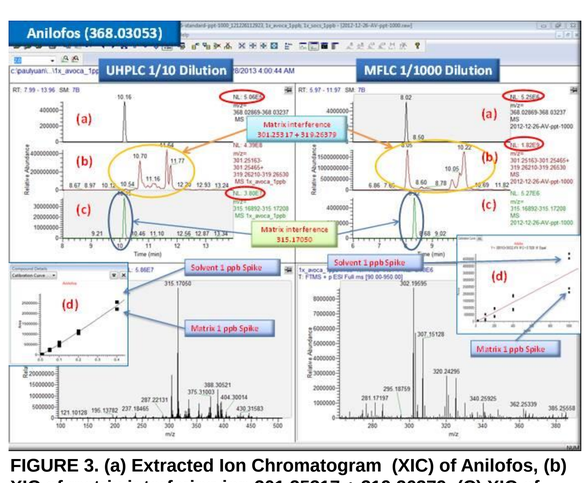

FIGURE 3. (a) Extracted Ion Chromatogram (XIC) of Anilofos, (b) XIC of matrix interfering ion 301.25317 + 319.26379, (C) XIC of matrix interfering ion 315.17050, (d) Calibration curve on matrix spiked samples.

The small matrix effect on UHPLC analysis is the result of the coeluted matrix ion of 315.17050 which is less than 75 times. The UHPLC analysis suffered with the same matrix interfering ion (301.25317 + 319.26379) in Figure 3 (b)but due to the different column and different LC system, the XIC of the target ion (368.03052) is not coeluted with those two matrix interfering ions. It is concluded that ion suppression during the ESI ionization process is a function of the ion ratio between interfering ions and the target ion, if it is great than 100 times. It can be verified through all experiment data of more than 300 data set.

In the event if interfering ions are perfectly coeluted with target ion, then the dilution will not give any advantage due to the ion suppression in the ESI ionization process which is suppressed on the ion ratio and it will not be changed by any dilution if perfectly coeluted condition exist, and It was proved through our entire experimental observation with more than 300 compounds and 9 different sample matrices with 4 different dilution factor (1X, 10X, 1ooX and 1000X). However, the high dilution may help skip the ion suppression if the interfering ions are not perfectly coeluted with the target ion then the dilution may narrow the peak of the interfering ion and reducing the ratio between interfering and target ion which can reduce or eliminate the matrix interference by this approach.

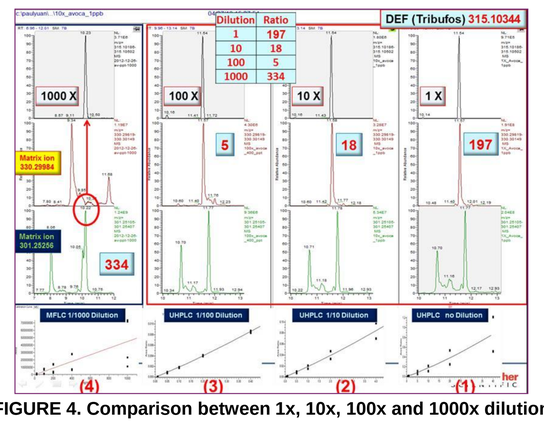

FIGURE 4. Comparison between 1x, 10x, 100x and 1000x dilution

The right 3 column (1), (2) and (3) were run on UHPLC on spinach sample matrix with 1x, 10x and 100 dilution then post spiked with 5 calibration levels according to the experimental section. The results is clearly showing that ion suppression or matrix effect is diminished after 100 time of dilution, this is consistence with most of the publication regarding dilution can avoid the matrix effect on sample run. However, when we had 1000x dilution which ran on MFLC with different column which showed serious ion suppression in column 4. After carefully examined the Extraction Ion chromatography (EIC) from all ion chromatogram and we found that due to the different system with different column and solvent gradient. The coelute ion in the UHPLC sample showing that the ration of interfering ion to the target ion dropped from 197/1 to 18/1 and 5/1 according to the dilution 1x , 10 x and 100x. The column 4 is 1000x sample run from MFLC, the interference ion is different than in the UHPLC samples and the ratio is 334/1. As we previously mention if the interference ion ratio is high than matrix effect will be severe, the only way to avoid the matrix effect is through the chromatography separation if it is perfectly coeluted together. In the event if it is not perfectly coeluted but with some overlapping then dilution will reduce the concentration on the matrix ion and minimized the ion suppression, meaning dilution may be able to solve the matrix effect as seen on the column (3) in Figure 4.

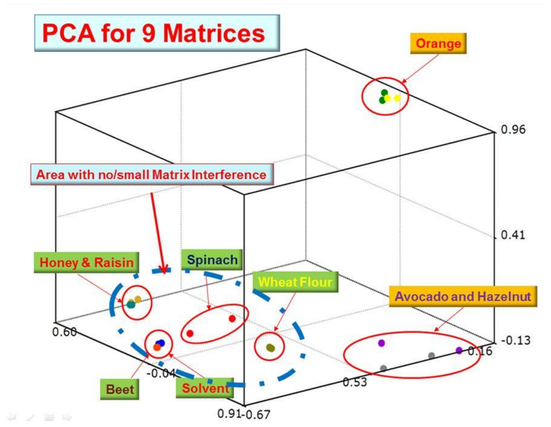

FIGURE 5. PCA on 9 sample matrices

All nine sample matrices were run through Principal Component Analysis(PCA) and results is shown in Figure 5., 381 pesticides were run on 9 difference sample matrices with 5 level of calibration curve. It is clearly that Spinach, Honey, Raisin, Beet, Wheat Flour are very close to solvent stand in Blue spot during PCA analysis. Also it is our finding that these 5 matrices had very little or no matrix effect compare to Avocado, Hazelnut and Orange samples.

Conclusions

If target ion is perfectly coeluted with matrix ion then dilution will not be the solution for ion suppression since the ion ratio will remain the same. Ion suppression can be avoided by dilution or different chromatography conditions (different column), if you only need to analyze few compounds. For multi-residue analysis, you can fix on some target compounds but not for all of them.

Be careful on choosing Internal standard if the matrix matched standard is not same as your sample, then the response will be different.

References

Tang, L.; Kebarle, P. Dependence of Ion Intensity in Electrospray Mass Spectrometry on the Concentration of the Analytes in the Electrosprayed Solution. *Anal. Chem.* **1993**, 65, 3654−3668.

Bruins, A. P. Mechanistic aspects of electrospray ionization. *J. Chromatogr. A*. **1998**, 794, 345−357.

Zhang, K.; Wong, J. W.; Hayward, D. G.; Vaclavikova, M.; Liao, C. D.; Trucksess, M. W. Determination of mycotoxins in milk-based products and infant formula using stable isotope dilution assay and liquid chromatography tandem mass spectrometry. *J. Agric. Food Chem*. **2013**, 61, 6265−6273.

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