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ARTICLE

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"Cross-Talk" in Scheduled Multiple Reaction Monitoring Caused by In-Source Fragmentation in Herbicide Screening with Liquid Chromatography Electrospray Tandem Mass Spectrometry

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ABSTRACT: Interferences, or "cross-talk", have been found for the liquid chromatorgraphy mass spectrometry (LC-MS) determination of chlorophenoxy acid (CPA) herbicides. The time-scheduled multiple reaction monitoring (MRM) of m/z 161.0→125.0 and 163.0→127.0 transitions were produced by 2,4-dichlorophenol and 2,4-dichlorophenoxyacetic acid. Although MRM reduces the possibility of false positives when two transitions for LC/MS-MS are used for quantification and qualitative confirmation, in the case of the structurally related CPAs here, false positives still occurred when using a mixture of standards to identify the residues. It was necessary to analyze pure individual standards to compare with the extracted retention times of the candidate CPAs in food samples.

KEYWORDS: liquid chromatography mass spectrometry, herbicides, cross talk

INTRODUCTION

Herbicides are used worldwide to control weed and insect-borne disease to increase the agricultural output. Because of their possible toxicities, their residue levels in food and environmental samples need to be monitored. Gas chromatography (GC) coupled with different detectors[1,2] has been used to analyze herbicides. However, the drawback with GC is that derivatization is often required, which can be time-consuming and labor intensive. In recent years, liquid chromatography/electrospray tandem mass spectrometry (LC/ESI-MS-MS) has become popular for pesticide and herbicide residue analysis.[3,4] Together with a single method for the multiresidue extraction such as the quick, easy, cheap, effective, rugged, and safe method (QuEChERS),[5] this offers the opportunity of fast screening herbicide residues in food matrices therefore increasing efficiency in food analysis laboratories. When using a triple quadrupole mass spectrometer, the precursor ion is scanned selectively through the first quadrupole (Q_1) and then fragmented in the second quadrupole (Q_2). The characteristic product ions are selectively monitored in the third quadrupole (Q_3). By using the retention time and two product ion ratio, the identities of the analytes can be positively confirmed in general. It is known that in-source fragmentation could occur for ESI-MS in the region before the Q_1 under the typical condition for ESI-MS. The fractions of analytes that undergo fragmentation are also known to be low. The cone voltage is intentionally elevated in these cases where single quadrupole mass spectrometer is used to get MS/MS like spectra, the elevated voltage fragments all ions without mass selection. In this work, significant in source fragmentations from phenoxy acids on the ABI 4000 Q-trap were observed under the normal condition used for herbicide analysis without deliberately increasing the cone potential. Here I report my results about this observation.

MATERIALS AND METHODS

Methanol as high purity solvent was obtained from Honeywell Burdick & Jackson (Muskegon, MI). Ammonium formate was obtained from Sigma-Aldrich (St. Louis, MO). All herbicide standards, namely, 2,4-dichlorophenoxy acetic acid (2,4-D), 2,4-dichlorobutyric acid (2,4-DB), 2,4-dichloroprop, 2,4-dichlorophenol, 2,4,5-trichlorophenoxy acetic acid (2,4,5-T), 2,4,5-trichlorobutyric acid (2,4,5-TB), and 2,4,5-trichlorophenol, clopyralid, trichloropyr, quinclorac, haloxyfop, mecoprop, dicamba, pentachlorophenol (PCP) were provided by the Environmental Protection Agency (Fort Meade, MD). The standard solution of individual standard (1 μg/mL) was prepared in CH_3OH:H_2O (v/v, 1/1). The standard mixture solutions which contained all above compounds (20−1000 ng/mL) were diluted with CH_3OH:H_2O (v/v, 1/1) from the stock solution in methanol.

The mass spectra were acquired on a Q-trap 4000 LC/MS-MS system (Applied Biosystems, Foster City, CA) equipped with a LC system from Shimadzu (Columbia, MD). The LC system consisted of two models of 20AD binary pump, a model of DGU-20A3 online degasser, a model of SIL-20AC autosampler. Gradient elution started with 4.0 mM ammonium formate and 0.1% formic acid in 1:9 CH_3OH:H_2O (v/v) and changed linearly over 8 min to 4 mM ammonium formate and 0.1% formic acid in 9:1 CH_3OH:H_2O (v/v). It was held at 4.0 mM ammonium formate and 0.1% formic acid in 9:1 CH_3OH:H_2O (v/v) for 6 min and then changed linearly to 4.0 mM ammonium formate and 0.1% formic acid in 1:9 CH_3OH:H_2O (v/v) within 1 min. The flow rate was 0.350 mL/min. The column was an Atlanta 3 μ, 2.1 × 100 mm C_{18} from Waters (Milford, MA). The column temperature was 40 °C. The injection volume was 3 μL.

The ESI-MS/MS was performed in scheduled multiple reaction monitoring (MRM) and also unscheduled MRM in the negative ion mode. Nitrogen gas was used as curtain gas (50 psi), sheath gas (40 psi), drying gas (50 psi), and collision gas. The source temperature for the turbo ion spray gas was 550 °C. The spray potential was −4500 eV. The collision energy and declustering energy were optimized using direct injection.

RESULTS AND DISCUSSION

A total of 14 different herbicides, as listed in the materials, mostly chlorophenoxy acids, were selected for developing a LC/MS-MS method for herbicide screening by using MS/MS parameters, the transitions and retention times listed in

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Table 1. MS/MS Parameters for Herbicide Detection by Scheduled MRM Method[a]

Q1 mass	Q3 mass	time (min)	transition ID	DE	EP	CE	CXE
252.9	195.0	8.7	2,4,5-T1	−20	−4.5	−20	−12
254.9	197.0	8.7	2,4,5-T2	−20	−4.5	−20	−12
218.9	161.0	8.1	2,4-D1	−25	−10	−20	−14
220.9	16.0	8.1	2,4-D2	−25	−10	−20	−14
247.0	161.0	8.9	2,4-DB1	−15	−4.5	−22	−14
249.0	163.0	8.9	2,4-DB2	−15	−4.5	−22	−14
189.9	145.9	4.6	clopyralid1	−15	−8	−10	−14
191.9	147.9	4.6	clopyralid2	−15	−8	−10	−14
219.0	175.0	7.2	dicamba1	−15	−4.5	−8	−14
221.0	177.0	7.2	dicamba2	−15	−4.5	−8	−14
213.0	141.0	8.6	mecoprop1	−25	−4.5	−22	−12
213.0	71.0	8.6	mecoprop2	−25	−4.5	−14	−9
264.8	35.1	10.0	PCP1	−50	−4.5	−42	−6
262.9	35.1	10.0	PCP2	−50	−4.5	−42	−6
253.9	196.0	8.6	triclopyr1	−15	−4.5	−16	−18
255.9	198.0	8.6	triclopyr2	−15	−4.5	−16	−18
239.9	195.9	7.1	quinclorac1	−45	−10	−10	−5
241.9	197.9	7.1	quinclorac2	−45	−10	−10	−5
281.0	195.0	9.3	2,4,5-TB1	−45	−10	−20	−5
283.1	197.0	9.3	2,4,5-TB2	−45	−10	−20	−5
197.0	161.0	9.0	2,4,5-trichlorophenol1	−45	−10	−20	−5
199.0	163.0	9.0	2,4,5-trichlorophenol2	−45	−10	−20	−5
360.0	288.0	9.2	haloxyfop1	−45	−10	−20	−5
362.0	290.0	9.2	haloxyfop2	−45	−10	−20	−5
161.0	125.0	8.2	2,4-dichlorophenol1	−45	−10	−20	−5
163.0	127.0	8.2	2,4-dichlorophenol2	−45	−10	−20	−5
233.0	161.0	8.6	2,4-dichloroprop1	−45	−10	−22	−5
233.0	125.0	8.6	2,4-dichloroprop2	−45	−10	−38	−5

[a] DE: declustering potential. EP: entrance potential. CE: collision energy. CXE: collision cell exit potential. Transition ID: the first transition of each compound is the quantifier, and the second one is the qualifier.

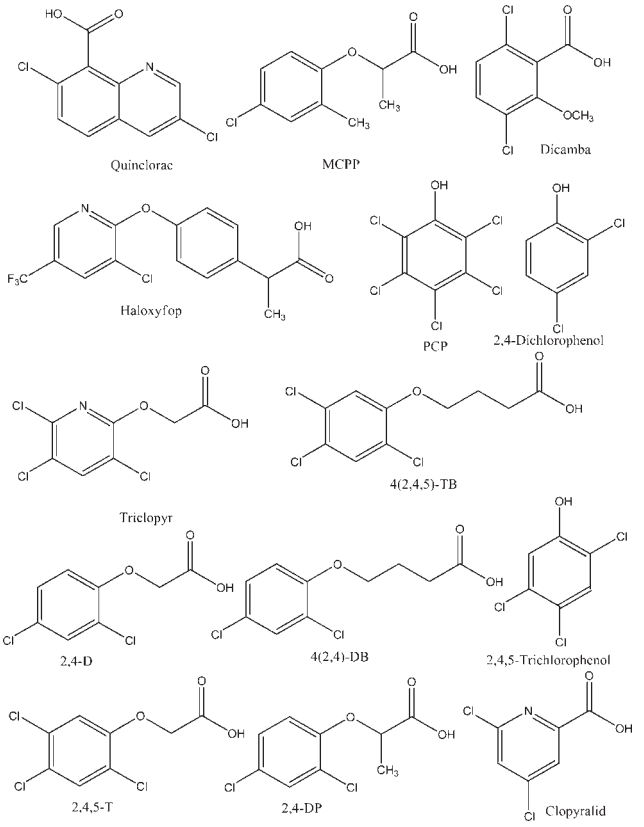

Figure 1. Chemical structures of herbicide compounds.

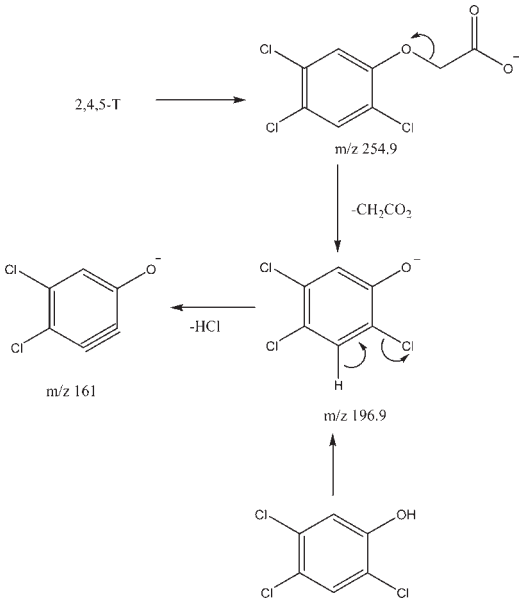

Figure 2. Proposed fragmentation pathway of 2,4,5-T and 2,4,5-trichlorophenol.

Table 1 and the structures are shown in Figure 1. Proposed fragmentation pathways are shown in Figure 2 and 3. To achieve the best detection limit and better quantification, time-scheduled MRM [4] was used, in which the software calculated the time windows for monitoring the transitions of the analytes near the

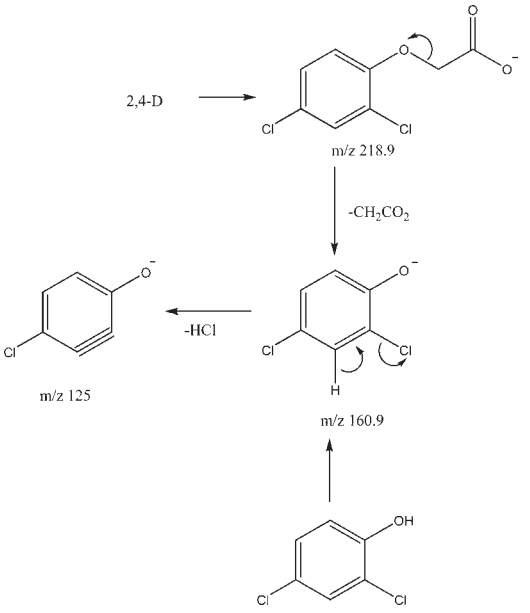

Figure 3. Proposed fragmentation pathway of 2,4-D and 2,4-dichlorophenol.

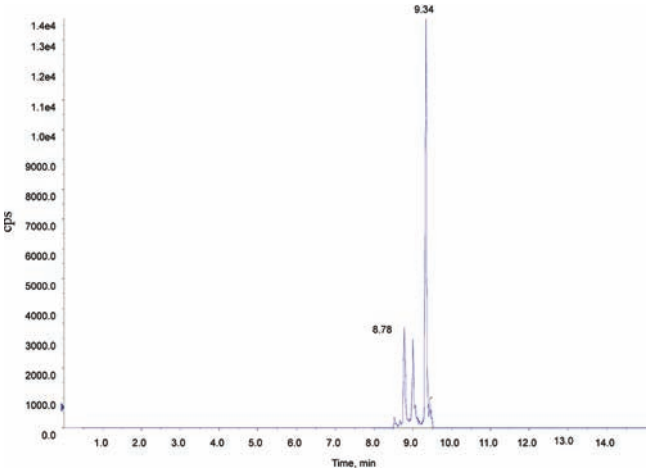

Figure 4. MRM (m/z 197.0→161.0) chromatogram of 2,4,5-trichlorophenol obtained from 1.0 μg/mL of the standard mixture in methanol/water.

expected LC elution times, which optimized the time spent on each MRM transition to obtain more data points. The expected retention times were obtained using unscheduled MRM, which scans all the transitions through the 15 min analysis under the same chromatographic and mass spectrometer conditions as used in the time scheduled MRM (as described in the experimental section).

While the extracted ion chromatograms of most of the herbicide compounds appeared as a single peak with only one retention time for each herbicide, the chlorinated phenols showed multiple peaks, as shown in Figure 4. Therefore, the correct one had to be identified by comparing with the retention time of the pure standard compound. The extracted ion chromatogram of 2,4,5-trichlorophenol showed three peaks eluting at 8.78, 8.99, and 9.34 min. A pure standard of 2,4,5-trichlorophenol showed a peak eluting at 9.00 min with transitions of m/z 197.0→161.0 and 199.0→163.0 for the second chlorine isotope (Figure 5). A chromatogram of pure standard of 2,4,5-T using

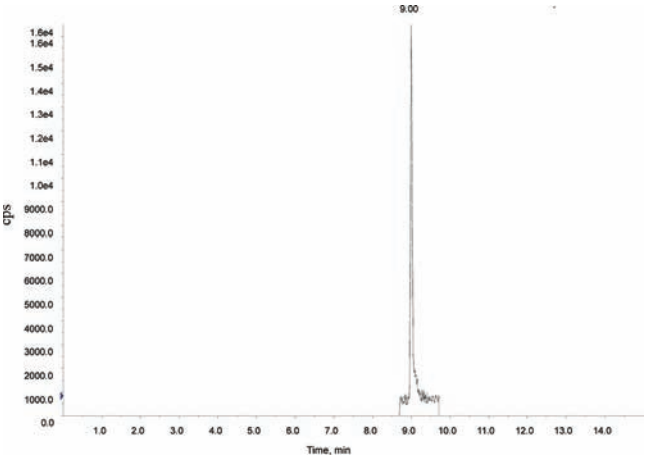

Figure 5. MRM (m/z 197.0→161.0) chromatogram of 2,4,5-trichlorophenol obtained from 1.0 μg/mL of the standard in methanol/water.

the scheduled MRM method also showed a peak eluting at 8.78 min for both of the transitions of 2,4,5-trichlorophenol above. The extracted ions with transitions of m/z of 199.0→163.0 and 197.0→161.0 from 2,4,5-T were also found with about an equally intensity at this retention time. The fragmentation of 2,4,5-T in negative ion mode can give the 2,4,5-trichlorobenzoxy anion in the source region, which further fragmented in the second quadrupole to give the benzyne anion after losing HCl (Figure 2). The fragmentation of 2,4,5-TB in the source region as above probably explained the observed peak at 9.34 min for the extracted ion of the anion of 2,4,5-trichlorophenol, and pure standard of 2,4,5-TB showed a peak at 9.34 min using extracted ion of 281.0/195.0 and 283.0/197.0. So, the presence of 2,4,5-T or 2,4,5-TB might also have been reported as false positives in the attempted detection of 2,4,5-trichlorophenol if the data have not been carefully cross-checked with pure reference standards.

The extracted ion chromatogram of 2,4-dichlorophenol was used in the analysis of the mixture of standards. It showed that two peaks eluted at 8.12 and 8.22 min (Figure 6a). That is, some of the standards other than 2,4-dichlorophenol produced the same transitions, even though they eluted off the column at different times. When compared to the retention time of the pure standard, the peak at 8.22 min was proven to be from 2,4-dichlorophenol. The observed peak eluting at 8.12 min, was probably from the in source fragmentation of 2,4-D. This fragmentation can give 2,4-dichlorophenol anion at m/z 161.0, which further fragmented in Q_2 to give a peak at m/z 125.0 (Figure 3). These were the same ions responsible for the observed MRM transition of 2,4-dichlorophenol. A peak at 8.64 min from 2,4-dichloroprop for both transitions was also observed when the expected retention time for both transitions were changed from 8.12 to 8.16 min where 2,4-D eluted when using pure standard. In this case, the 2,4-D and 2,4-dichlorophenol were not resolved (Figure 6b). It probably followed a very similar fragmentation pathway as 2,4-D via an 2,4-dichlorophenol anion intermediate to give both transitions.

Cross talk due to incomplete clearance of product ions in the Q_3 from the first monitored compound before the measurements of the second transition from the second compound has been reported, which could lead to false positives when two analytes have the same product ions.[6] Our results showed that a different "cross talk" could also occur. It was caused by in-source fragmentation when structurally related compounds were analyzed by LC/MS-MS. MRM reduced the possibility of false positives when two transitions were used for quantification and qualitative confirmation. In the case of structurally

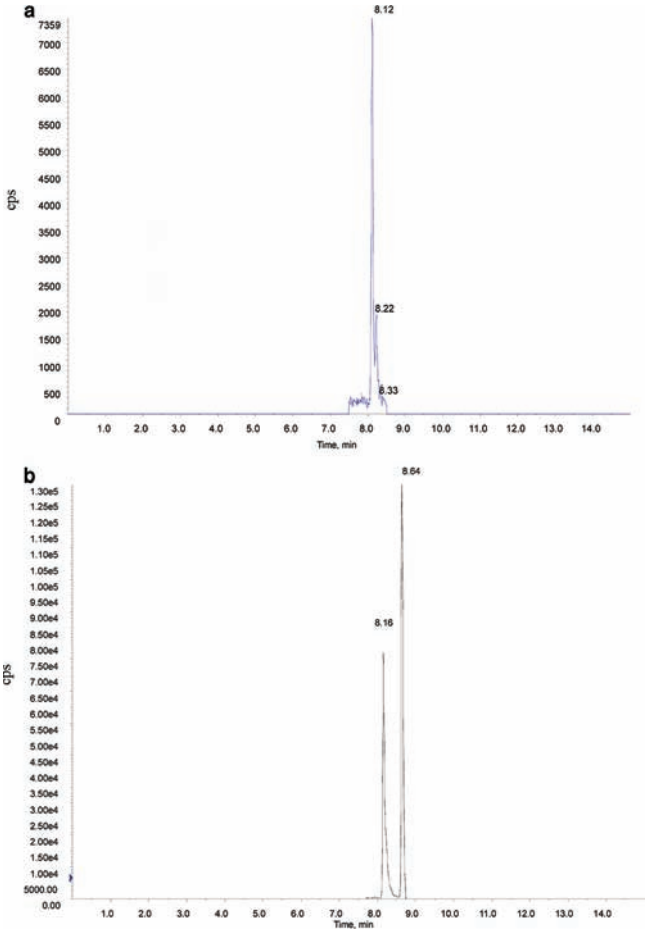

Figure 6. (a) MRM (m/z 161.0→125.0) chromatogram of 2,4-dichlorophenol obtained from 0.5 μg/mL of the standard mixture in methanol/water (7.66−8.62 min scan window). (b) MRM (m/z 161.0→125.0) chromatogram of 2,4-dichlorophenol obtained from 1.0 μg/mL of the standard mixture in methanol/water (7.66−8.66 min scan window).

related compounds here, false positives could still occur when using the commercially available standard mixture to identify the residues. It was necessary to chromatograph pure individual standards and compare the chromatograms to the extracted chromatograms of the analytes in the sample.

By use of the transitions with corrected retention times in Table 1, spectra of the standard mixture at different concentration levels were obtained from 20 to 1000 ng/mL. The peaks for different analytes were extracted and integrated. The results showed good linearity with R^2 better than 0.99 for most of the standards, except pentachlorophenol (0.96), and dichlorophenol was not resolved from 2,4-D in this experiment.

In conclusion, analysis of individual standard by LC/MS-MS was needed to confirm the assignment of the phenoxy acid herbicides in the method. Extracted ion chromatograms from the standard mixture alone could cause false positives, because of the significant in-source fragmentation from structurally related herbicides.

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