

Determination of Routinely Monitored Herbicides in Drinking Water Samples Using Automated Solid Phase Extraction Prior to Liquid Chromatography/Tandem Mass Spectrometry

Most countries have regulations or monitoring programs to research and track the level of herbicide contaminants in drinking water. Many populations obtain drinking water from ground water supplies, and it is these supplies that are at potential contamination risk from leaching contaminants, such as herbicides.¹ The US EPA has implemented an exposure research program aimed at conducting drinking water research on methods as part of the Microbiological and Chemical Exposure Assessment. This research is aimed at evaluating the chemical pollutants and their role and levels in which there is an unacceptable risk to either humans or wildlife, as well as evaluating the methodologies currently being used to determine levels of chemical pollutants.²

“Automated solid phase extraction provides excellent recoveries for these herbicide compounds and compound metabolites.”

Acetochlor is one of the most common active ingredients found in herbicides containing chloroacetanilide, and while acetochlor rapidly degrades in soils, routine studies determine the levels of leaching through different soil types and into the ground water supplies across a range of regions.³ The roots and leaves of emerging weeds absorb the acetochlor in order to prevent proteins from being synthesized. Alachlor is another contaminant highly researched. It is listed as a regulated contaminant under the US Safe Drinking Water Act at a maximum contaminant level of 0.002 mg/L.⁹ Several of the other analytes listed in Table 1 are on the contaminant list for unregulated contaminant monitoring rule 3 (UCMR-3), and are often tested during routine monitoring studies for occurrence determination.⁸

Global consumers remain concerned about the levels of common contaminants found in drinking water⁷ NSF International, a worldwide public health and safety organization, has investigated home water treatment options that are now widely available to consumers and are certified to NSF/ANSI 401 at varying levels. NSF has certified 56 filtration devices that have been tested for water treatment and reduce levels of up to 15 contaminants commonly found in drinking water, including Metolachlor, a commonly detected herbicide.⁶

Herbicides in Europe are regulated by monitoring the individual compounds under Council Directive 98/83/EC.⁴ Each herbicide detected cannot be present at greater than 0.1 µg/L or a total of all herbicides cannot exceed 0.5 µg/L. Herbicides are part of a larger pesticides category of monitored contaminants that track levels of insecticides, herbicides, fungicides, and related categories.

Traditional testing of herbicides in water samples uses SPE to concentrate and selectively cleanup samples prior to evaporation and analysis. This SPE process is commonly performed manually using a vacuum manifold to pull the sample through the sorbent; however, enabling automation to perform the manual SPE procedure can increase the efficiency of a laboratory's work flow and allow other technician activities to be accomplished during the SPE process. A research study was performed to determine the levels of chloroacetanilide and chloroacetanilide metabolites (CAAMs) in laboratory water samples using automation for the SPE concentration step prior to LC/MS/MS analysis against a 7-point calibration curve. The SPE method used for this laboratory work was modified from the regulatory EPA 535 method. Generated recovery and reproducibility results were compared against the acceptance criteria listed by the EPA 535 method.

Materials & Methods:

Reagents

All reagents used were laboratory grade.

- Reagent water
- Methanol
- Ammonium acetate
- Purified nitrogen

Spiking Compounds

Purified laboratory water samples (250 mL) were spiked at 2.5 ng/mL in triplicate with the suite of 12 compounds listed in Table 1.⁵

Herbicide Compound Name	CAS Registry Number
Acetochlor	34256-82-1
Acetochlor OA	184992-44-4
Acetochlor ESA	187022-11-3
Alachlor	15972-60-8
Alachlor OXA	171262-17-2
Alachlor ESA	142363-53-9
Dimethenamid	87674-68-8
Dimethenamid OA	30667
Dimethenamid ESA	205939-58-8
Metolachlor	51218-45-2
Metolachlor OA	152019-73-3
Metolachlor ESA	171118-09-5

Table 1. Suite of 12 herbicide compounds used for spiking.

Solid Phase Extraction (SPE) System & Method:

- SPE Cartridges: Supelclean ENVI-Carb SPE cartridges, 0.5 g, 6 mL contains a nonporous graphitized carbon sorbent phase
- SPE System: SmartPrep Extractor by Horizon Technology
- SPE Method: Figure 1

Extract Concentration:

The sample eluent extract was concentrated to dryness in a heated water block (50-60 °C). The primary dilution standard (PDS) was added as internal standard and 5 mM ammonium acetate/reagent water was added to a 1 mL final volume.

Author/Contact Details:

Toni Hofhine

Horizon Technology, Inc.,

16 Northwestern Drive, Salem, NH USA

Tel: +1.603.893.3663

Email: sales@horizontechinc.com

Web: www.horizontechinc.com

Peter Ma, Marie Assem, Terry Cain,
Lynne Sullivan, and Michael Wichman
The State Hygienic Laboratory
University of Iowa, Coralville, IA USA

Determination of Routinely Monitored Herbicides in Drinking Water Samples Using Automated Solid Phase Extraction Prior to Liquid Chromatography/Tandem Mass Spectrometry

Most countries have regulations or monitoring programs to research and track the level of herbicide contaminants in drinking water. Many populations obtain drinking water from ground water supplies, and it is these supplies that are at potential contamination risk from leaching contaminants, such as herbicides.¹ The US EPA has implemented an exposure research program aimed at conducting drinking water research on methods as part of the Microbiological and Chemical Exposure Assessment. This research is aimed at evaluating the chemical pollutants and their role and levels in which there is an unacceptable risk to either humans or wildlife, as well as evaluating the methodologies currently being used to determine levels of chemical pollutants.²

“Automated solid phase extraction provides excellent recoveries for these herbicide compounds and compound metabolites.”

Acetochlor is one of the most common active ingredients found in herbicides containing chloroacetanilide, and while acetochlor rapidly degrades in soils, routine studies determine the levels of leaching through different soil types and into the ground water supplies across a range of regions.³ The roots and leaves of emerging weeds absorb the acetochlor in order to prevent proteins from being synthesized. Alachlor is another contaminant highly researched. It is listed as a regulated contaminant under the US Safe Drinking Water Act at a maximum contaminant level of 0.002 mg/L.⁹ Several of the other analytes listed in Table 1 are on the contaminant list for unregulated contaminant monitoring rule 3 (UCMR-3), and are often tested during routine monitoring studies for occurrence determination.⁸

Global consumers remain concerned about the levels of common contaminants found in drinking water⁷ NSF International, a worldwide public health and safety organization, has investigated home water treatment options that are now widely available to consumers and are certified to NSF/ANSI 401 at varying levels. NSF has certified 56 filtration devices that have been tested for water treatment and reduce levels of up to 15 contaminants commonly found in drinking water, including Metolachlor, a commonly detected herbicide.⁶

Herbicides in Europe are regulated by monitoring the individual compounds under Council Directive 98/83/EC.⁴ Each herbicide detected cannot be present at greater than 0.1 µg/L or a total of all herbicides cannot exceed 0.5 µg/L. Herbicides are part of a larger pesticides category of monitored contaminants that track levels of insecticides, herbicides, fungicides, and related categories.

Traditional testing of herbicides in water samples uses SPE to concentrate and selectively cleanup samples prior to evaporation and analysis. This SPE process is commonly performed manually using a vacuum manifold to pull the sample through the sorbent; however, enabling automation to perform the manual SPE procedure can increase the efficiency of a laboratory's work flow and allow other technician activities to be accomplished during the SPE process. A research study was performed to determine the levels of chloroacetanilide and chloroacetanilide metabolites (CAAMs) in laboratory water samples using automation for the SPE concentration step prior to LC/MS/MS analysis against a 7-point calibration curve. The SPE method used for this laboratory work was modified from the regulatory EPA 535 method. Generated recovery and reproducibility results were compared against the acceptance criteria listed by the EPA 535 method.

Materials & Methods:

Reagents

All reagents used were laboratory grade.

- Reagent water
- Methanol
- Ammonium acetate
- Purified nitrogen

Spiking Compounds

Purified laboratory water samples (250 mL) were spiked at 2.5 ng/mL in triplicate with the suite of 12 compounds listed in Table 1.⁵

Herbicide Compound Name	CAS Registry Number
Acetochlor	34256-82-1
Acetochlor OA	184992-44-4
Acetochlor ESA	187022-11-3
Alachlor	15972-60-8
Alachlor OXA	171262-17-2
Alachlor ESA	142363-53-9
Dimethenamid	87674-68-8
Dimethenamid OA	30667
Dimethenamid ESA	205939-58-8
Metolachlor	51218-45-2
Metolachlor OA	152019-73-3
Metolachlor ESA	171118-09-5

Table 1. Suite of 12 herbicide compounds used for spiking.

Solid Phase Extraction (SPE) System & Method:

- SPE Cartridges: Supelclean ENVI-Carb SPE cartridges, 0.5 g, 6 mL contains a nonporous graphitized carbon sorbent phase
- SPE System: SmartPrep Extractor by Horizon Technology
- SPE Method: Figure 1

Extract Concentration:

The sample eluent extract was concentrated to dryness in a heated water block (50-60 °C). The primary dilution standard (PDS) was added as internal standard and 5 mM ammonium acetate/reagent water was added to a 1 mL final volume.

Author/Contact Details:

Toni Hofhine

Horizon Technology, Inc.,

16 Northwestern Drive, Salem, NH USA

Tel: +1.603.893.3663

Email: sales@horizontechinc.com

Web: www.horizontechinc.com

Peter Ma, Marie Assem, Terry Cain,
Lynne Sullivan, and Michael Wichman
The State Hygienic Laboratory
University of Iowa, Coralville, IA USA

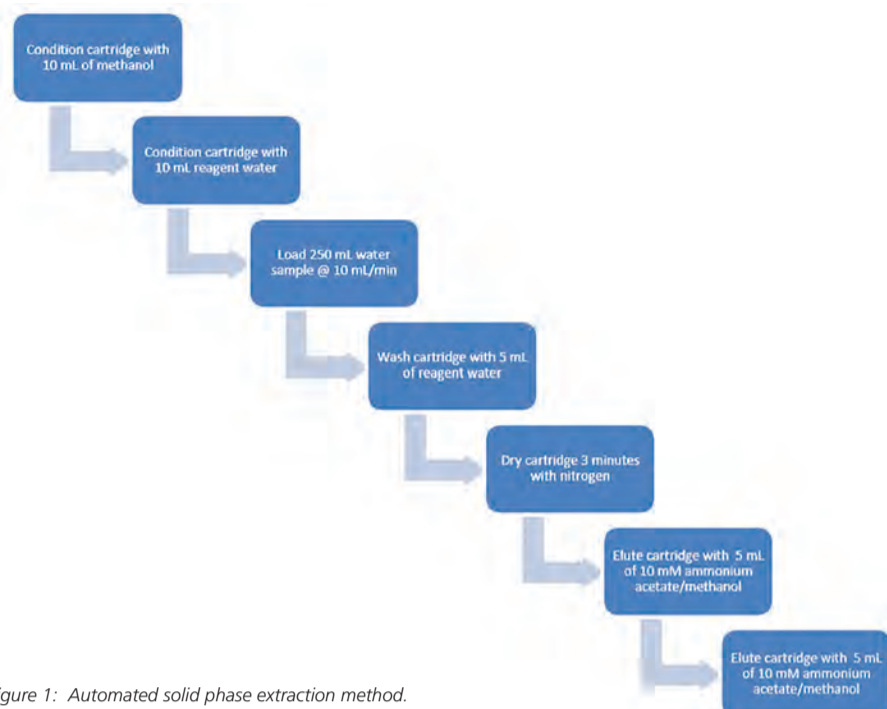


Figure 1: Automated solid phase extraction method.

LC/MS/MS Analysis:

- System: Waters Micromass Quattro Mass Spectrometer
- Column: Agilent Zorbax SB-C8, 3.0-mm X 100mm, dp= 5 µm
- Pre-Column: Upchurch pre-column filter, 0.5µm frit
- HPLC System: Waters Alliance Model 2690
- Injection Volume: 50 µL

Results:

Calibration standards were injected before and after water sample spikes. A 7-point calibration curve was used with levels ranging from 200 ppb to 0.6 ppb, with Alachlor OXA linearity results at 0.9996 (Figure 2). An example calibration standard chromatogram and mass spectra is shown in Figure 3. An example water sample spike chromatogram and mass spectra is shown in Figure 4. Blank injections were performed as part of the injection sequence, with herbicide compound levels lower than what could be quantitated.

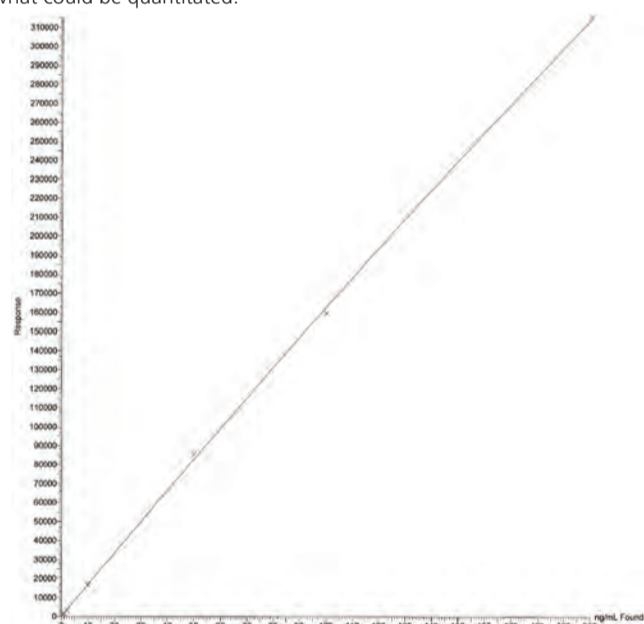


Figure 2: Example 7-point calibration curve for Alachlor OXA.

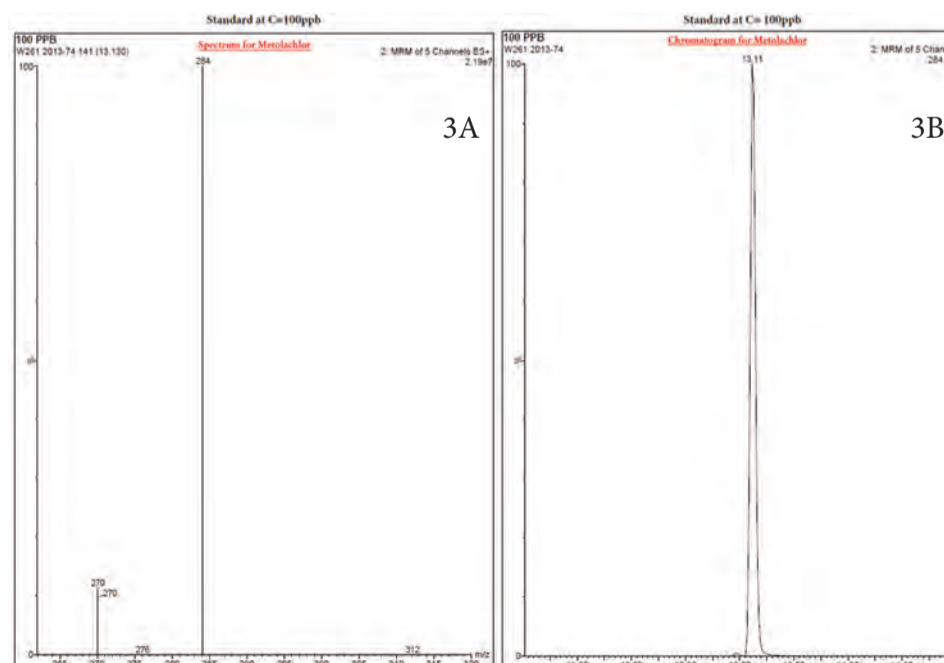


Figure 3A & 3B: Example calibration Metolachlor standard mass spectrum (3A) and chromatogram (3B).

Recoveries were calculated for the full suite of 12 compounds. Average recoveries from the triplicate water samples were reported, as well as %RSD values (Table 2).

Herbicide Compound Name	Mean Recovery (n=3)	% RSD (n=3)
Acetochlor	111	5.8
Acetochlor OXA	112	9.6
Acetochlor ESA	132	7.7
Alachlor	111	5.9
Alachlor OXA	108	10.7
Alachlor ESA	135	9.6
Dimethenamid	101	4.6
Dimethenamid OXA	102	8.2
Dimethenamid ESA	96	5.8
Metolachlor	98	5.9
Metolachlor OXA	99	11.7
Metolachlor ESA	111	9.4

Table 2: Calculated mean recoveries and %RSD values for spiked water

Conclusion:

Recovery determination of a suite containing 12 chloroacetanilide and chloroacetanilide metabolites can utilize simple automation to concentrate the SPE step for determination of levels within regulatory limits for drinking water samples. Automated solid phase extraction provides excellent recoveries for these herbicide compounds and compound metabolites. In addition, automation of the extraction procedure provided less technician intervention, creating a more efficient laboratory workflow, resulting in more reproducible performance.

The use of the SmartPrep Extractor to concentrate and cleanup water samples produced LC/MS/MS results that passed the requirements for mean recovery of water samples being 50-150% of the true spiked value of each of the 12 herbicide compounds, as well as passing the requirements for meeting the <20% RSD criteria for the precision criteria of the same water samples. Resulting recoveries ranged from 96 – 135%, with reproducibility ranging from 5.8% to 11.7%. Method detection levels were at 0.6 ppb. Blank injections resulted in no quantifiable herbicide compounds to report.

References:

1. Tatarikova, V., Hiller, E., and Halko, R. (2014): Retention Characteristics of Acetochlor in Soils Collected from Different Depths in Relation to Soil Properties, *Soil & Water Res.*, 9, (2): 58–65.
2. US EPA Drinking Water Methods Developed by EPA's Exposure Research Program (<http://www.epa.gov/nerlcwww/ordmeth.htm>), last visited on October 10, 2014.
3. Tomlin C.D.S. (2001): *The E-pesticide Manual*. Version 2.0. 12th Ed. British Crop Protection Council, [CD-ROM].
4. Official Journal of the European Communities (1998): Council Directive 98/83/EC, L 330/32.
5. US EPA Method 535, Measurement of Chloroacetanilide and Other Acetamide Herbicide Degradates in Drinking Water by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS), Version 1.1, April 2005, J.A. Shoemaker and M.V. Bassett, National Exposure Research Laboratory.
6. Water-Technology website: (<http://www.water-technology.net/contractors/wastewater/-nsf-international-global-testing/pressnsf-develops-water-treatment-device-standard.html>).
7. NSF website: (<http://www.nsf.org/newsroom/emerging-contaminants-in-driking-water-cause-consumer-health-concerns/>), last visited on October 10, 2014.
8. Environmental Protection Agency, 40 CFT Part 141, EPA-HW-OW-2012-0155; FRL-9917-87-OW, Announcement of Preliminary Regulatory Determinations for Contaminants on the Third Drinking Water Contaminant Candidate List.
9. Environmental Protection Agency, Reregistration Eligibility Decision (RED), EPA 738-R-98-020, December 1998.

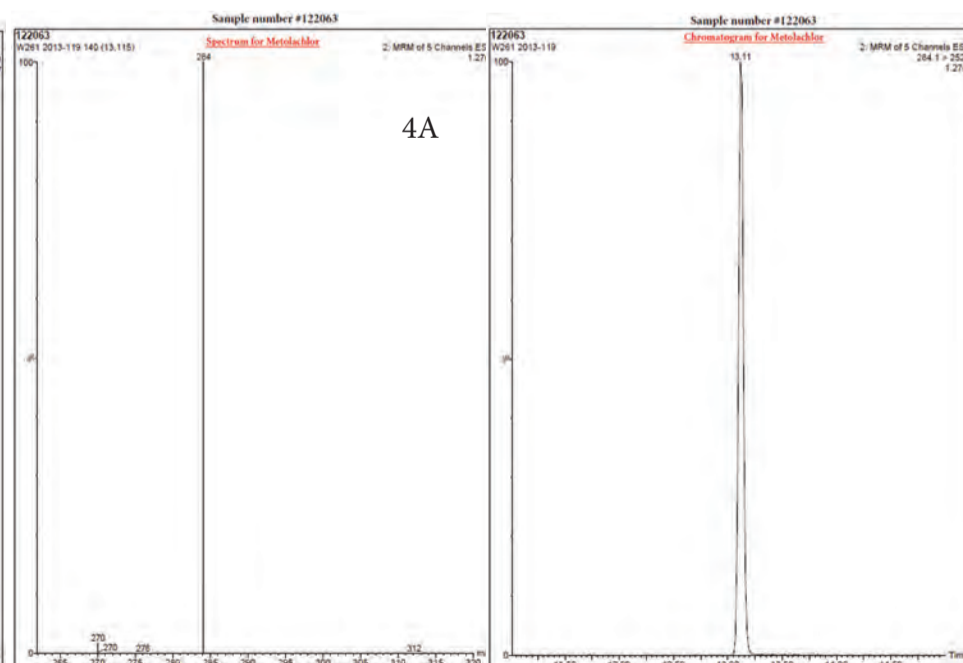


Figure 4A & 4B: Example spiked water sample spike mass spectrum (4A) and chromatogram (4B).

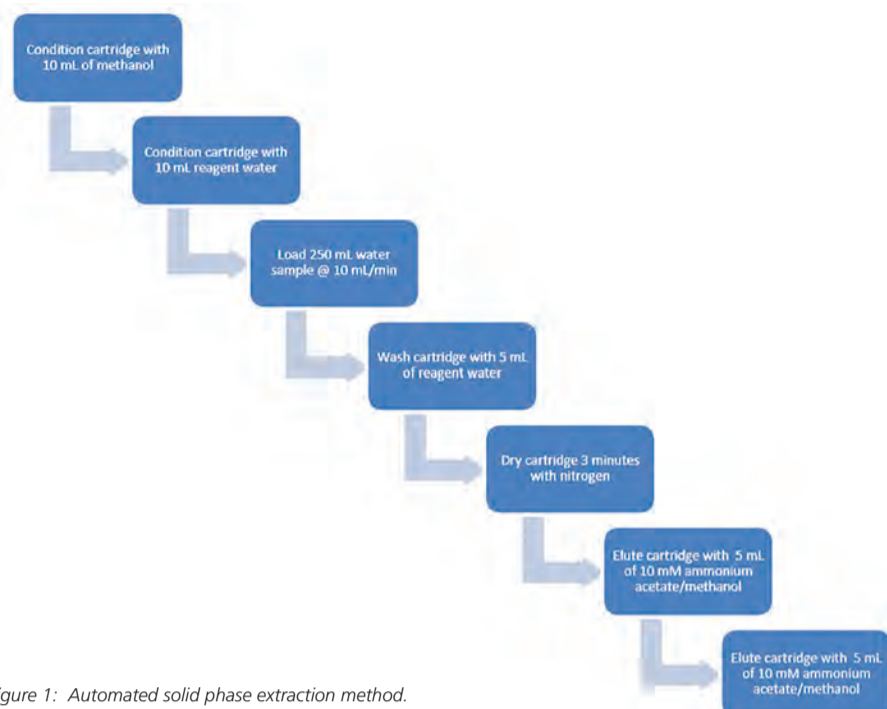


Figure 1: Automated solid phase extraction method.

LC/MS/MS Analysis:

- System: Waters Micromass Quattro Mass Spectrometer
- Column: Agilent Zorbax SB-C8, 3.0-mm X 100mm, dp= 5 µm
- Pre-Column: Upchurch pre-column filter, 0.5µm frit
- HPLC System: Waters Alliance Model 2690
- Injection Volume: 50 µL

Results:

Calibration standards were injected before and after water sample spikes. A 7-point calibration curve was used with levels ranging from 200 ppb to 0.6 ppb, with Alachlor OXA linearity results at 0.9996 (Figure 2). An example calibration standard chromatogram and mass spectra is shown in Figure 3. An example water sample spike chromatogram and mass spectra is shown in Figure 4. Blank injections were performed as part of the injection sequence, with herbicide compound levels lower than what could be quantitated.

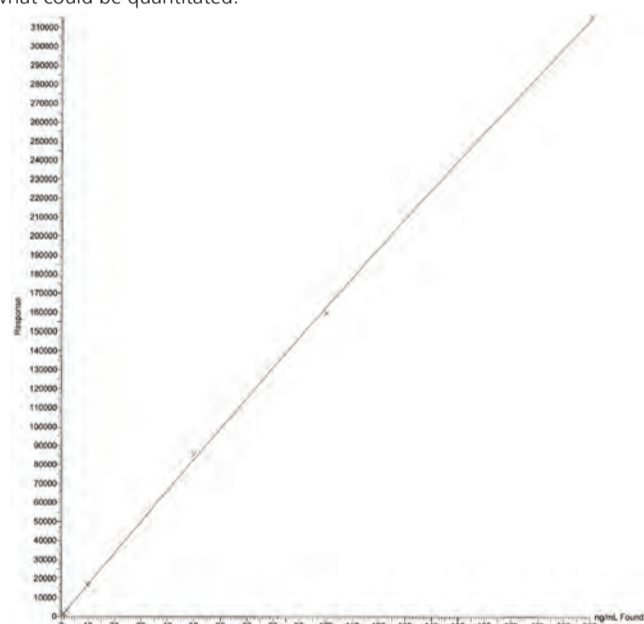


Figure 2: Example 7-point calibration curve for Alachlor OXA.

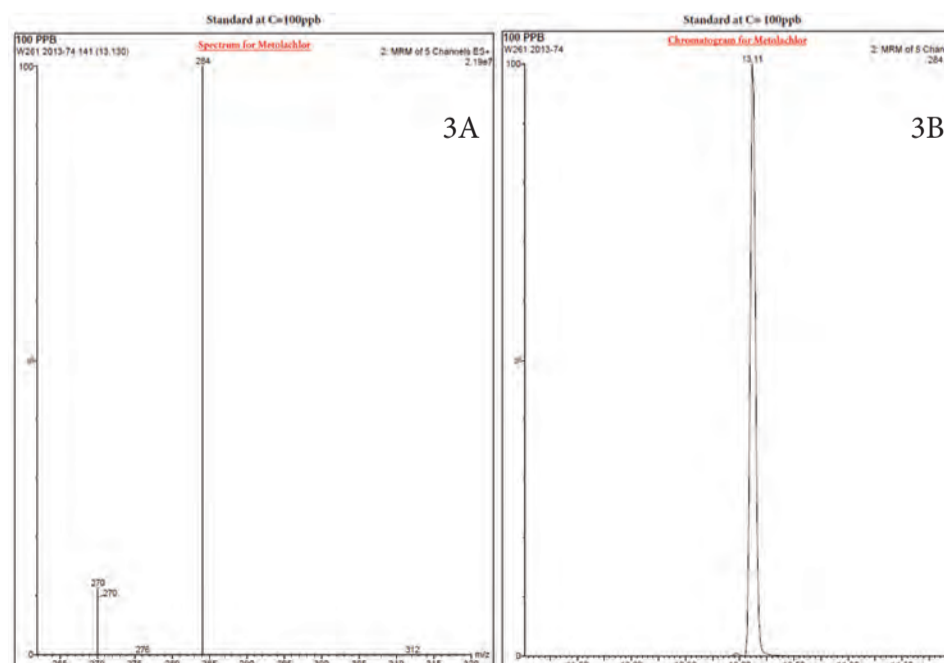


Figure 3A & 3B: Example calibration Metolachlor standard mass spectrum (3A) and chromatogram (3B).

Recoveries were calculated for the full suite of 12 compounds. Average recoveries from the triplicate water samples were reported, as well as %RSD values (Table 2).

Herbicide Compound Name	Mean Recovery (n=3)	% RSD (n=3)
Acetochlor	111	5.8
Acetochlor OXA	112	9.6
Acetochlor ESA	132	7.7
Alachlor	111	5.9
Alachlor OXA	108	10.7
Alachlor ESA	135	9.6
Dimethenamid	101	4.6
Dimethenamid OXA	102	8.2
Dimethenamid ESA	96	5.8
Metolachlor	98	5.9
Metolachlor OXA	99	11.7
Metolachlor ESA	111	9.4

Table 2: Calculated mean recoveries and %RSD values for spiked water

Conclusion:

Recovery determination of a suite containing 12 chloroacetanilide and chloroacetanilide metabolites can utilize simple automation to concentrate the SPE step for determination of levels within regulatory limits for drinking water samples. Automated solid phase extraction provides excellent recoveries for these herbicide compounds and compound metabolites. In addition, automation of the extraction procedure provided less technician intervention, creating a more efficient laboratory workflow, resulting in more reproducible performance.

The use of the SmartPrep Extractor to concentrate and cleanup water samples produced LC/MS/MS results that passed the requirements for mean recovery of water samples being 50-150% of the true spiked value of each of the 12 herbicide compounds, as well as passing the requirements for meeting the <20% RSD criteria for the precision criteria of the same water samples. Resulting recoveries ranged from 96 – 135%, with reproducibility ranging from 5.8% to 11.7%. Method detection levels were at 0.6 ppb. Blank injections resulted in no quantifiable herbicide compounds to report.

References:

1. Tatarikova, V., Hiller, E., and Halko, R. (2014): Retention Characteristics of Acetochlor in Soils Collected from Different Depths in Relation to Soil Properties, *Soil & Water Res.*, 9, (2): 58–65.
2. US EPA Drinking Water Methods Developed by EPA's Exposure Research Program (<http://www.epa.gov/nerlcwww/ordmeth.htm>), last visited on October 10, 2014.
3. Tomlin C.D.S. (2001): *The E-pesticide Manual*. Version 2.0. 12th Ed. British Crop Protection Council, [CD-ROM].
4. Official Journal of the European Communities (1998): Council Directive 98/83/EC, L 330/32.
5. US EPA Method 535, Measurement of Chloroacetanilide and Other Acetamide Herbicide Degradates in Drinking Water by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS), Version 1.1, April 2005, J.A. Shoemaker and M.V. Bassett, National Exposure Research Laboratory.
6. Water-Technology website: (<http://www.water-technology.net/contractors/wastewater/-nsf-international-global-testing/pressnsf-develops-water-treatment-device-standard.html>).
7. NSF website: (<http://www.nsf.org/newsroom/emerging-contaminants-in-driking-water-cause-consumer-health-concerns/>), last visited on October 10, 2014.
8. Environmental Protection Agency, 40 CFT Part 141, EPA-HW-OW-2012-0155; FRL-9917-87-OW, Announcement of Preliminary Regulatory Determinations for Contaminants on the Third Drinking Water Contaminant Candidate List.
9. Environmental Protection Agency, Reregistration Eligibility Decision (RED), EPA 738-R-98-020, December 1998.

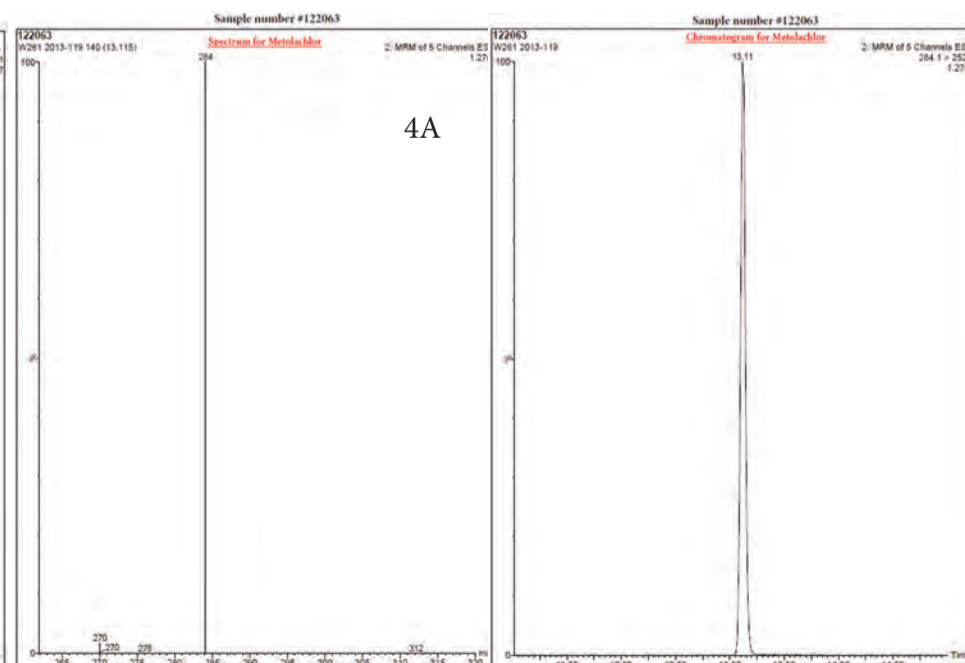


Figure 4A & 4B: Example spiked water sample spike mass spectrum (4A) and chromatogram (4B).