The Detection of Acidic Herbicides and Phenyl Ureas by LC-MS/MS with Large Volume Injection and Automated Column Switching

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Introduction

Acidic herbicides like Dicamba are used to kill broadleaf weeds before and after sprout. They control annual and perennial weeds in grain crops and highlands, are used to control brush and bracken in pastures, and in combination they are also used in pastures, range land, and noncrop areas (fence rows, roadways, and wastage) to control weeds. Phenyl urea pesticides such as Linuron are used as selective herbicides for pre- and post-emergence weed control in vegetables including potatoes, peas, carrots and beans; also on wheat, celery, parsnip and parsley. Both classes of pesticides are toxic to wildlife and some are suspected hormone-disrupting substances.

The provision of clean, uncontaminated drinking water is of paramount importance to the water industry. In recent times the requested limits of detection for such pesticides have been decreasing as methodologies improve. Typically water companies need to be able to have limits of quantitation for pesticides between 0.1 – 1 µg/L (100 – 1000 part-per-trillion, ppt) which often means that methods should have limits of detection for certain pesticides in the range of 10 – 50 µg/L.

These low levels have often meant that water samples have to be prepared either by liquid/liquid or solid phase extraction in order to concentrate these contaminants to such a level where they can be detected using traditional techniques such as GC-MS or HPLC with UV detection. Where GC-MC is used an additional derivatization step is often required before sample analysis. This sample pre-treatment used for traditional techniques can often be time consuming and add additional cost to the analyses. Therefore in this work the direct injection of filtered samples was used for sample analysis, to reduce both cost and speed up the sample throughput.

Experimental

Sample Preparation

River and ground water samples (10 mL) were filtered through a Chromfil PET 20/25, 0.2 µm 25 mm filter. The filter was washed by acetonitrile (0.85 mL) with the filter wash added directly into the sample. This filtered sample was directly injected onto the LC-MS/MS system.

Chromatography

Samples (200 µL) were directly injected and separated by reversed-phase HPLC using a Dionex Ultimate 3000 system. A Gemini 3 µm, 150 x 2.0 mm C18 and a LUNA 3 µm C18 (2), 150 x 3 mm column from Phenomenex were used to analyze acid herbicides and phenyl ureas respectively. Both columns were kept at 40ºC and gradients from water containing 0.1% acetic acid to acetonitrile containing 0.1% acetic acid were used to separate analytes. Automated column switching, involving a 10 port Valco switching valve, was used to switch between the column for acidic herbicide and the one for phenyl urea analysis (the gradient profiles are shown in Table 1).

Table 1. Gradient profiles used for the separation of acidic herbicides and phenyl ureas

Acidic herbicides			Phenyl ureas		
Time (min)	Flow (mL/min)	% B	Time (min)	Flow (mL/min)	% B
0.0	10	0.4	0.0	10	0.2
1.5	10	0.4	5.0	10	0.2
10.0	95	0.7	9.0	100	0.3
18.0	95	0.7	16	100	0.3
18.5	10	0.4	17	10	0.2
18.6	10	0.4			

Mass Spectrometry

Analysis was performed on an AB SCIEX API 4000™ LC/MS/MS system with Turbo V™ source electrospray ionisation (ESI) probe in negative polarity (acidic herbicides) and positive polarity (phenyl ureas). The MRM transitions for acidic herbicides and phenyl ureas are shown in Table 2.

Results and Discussion

Examples of calibrations for both acidic herbicides and phenyl ureas are shown in Figures 1, 2 and 3. For both classes of pesticides linear responses were obtained over the range tested with 'r' values never less than 0.998 (Table 3).

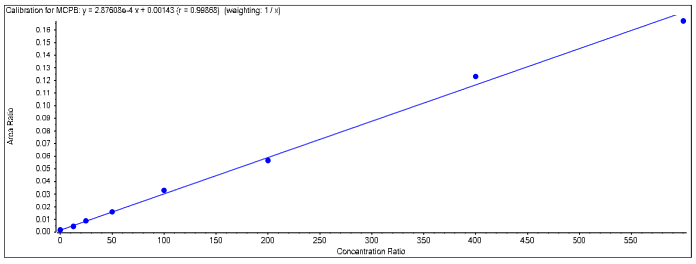

Figure 1. Calibration for MCPB from 12.5 – 600 ng/L

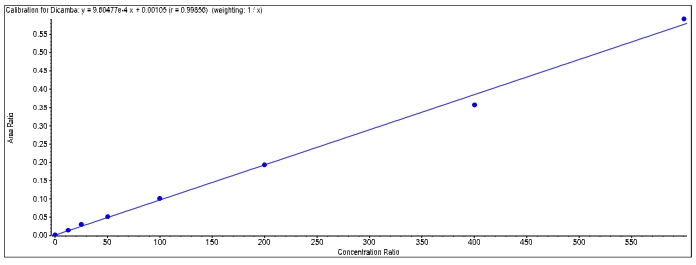

Figure 2. Calibration for Dicamba from 12.5 – 600 ng/L

Table 2. MRM transitions to detect acidic herbicides and phenyl ureas using the AB SCIEX API 4000™ system

Pesticide	Q1	Q3	DP	CE
MCPA	199.0	141.1	-55	-20
Clopyralid A	189.9	146.0	-20	-12
Clopyralid B	191.9	148.0	-20	-12
2,4-D	218.9	161.1	-20	-20
Dicamba	218.9	175.1	-20	-8
2,4-DB	246.9	161.0	-20	-18
Dichlorprop	232.9	161.1	-25	-18
Bromoxynil	275.8	81.0	-50	-45
Ioxynil	369.7	127.0	-55	-50
Bentazone	239.0	132.0	-50	-36
MCPB	227.1	141.1	-35	-25
MCPP	213.0	141.1	-30	-22
Triclopyr	253.9	196.0	-20	-16
Fluroxypyr	253.0	195.0	-35	-20
Benazolin	242.0	170.1	-25	-20
Aminopyralid	204.8	160.8	-55	-14
2,4-DPA (S)	203.1	159.1	-35	-12
4-CAA (IS)	169.0	125.0	-20	-12
2,B-4,C-phenol (IS)	195.9	78.9	-45	-32
Isoproturon A	207.1	134.2	45	35
Isoproturon B	207.0	72.0	56	35
Diuron	233.0	72.0	71	35
Isoproturon	207.0	72.0	56	35
Monolinuron	215.0	126.0	56	25
Chlorotoluron A	215.0	182.9	51	11
Chlorotoluron B	213.0	72.0	51	15
Metoxuron	229.0	72.0	106	35
Fenuron	165.2	72.0	86	29
Pencycuron	329.0	124.8	90	39
Linuron	249.0	159.9	51	27
Isoproturon	207.1	134.2	45	35

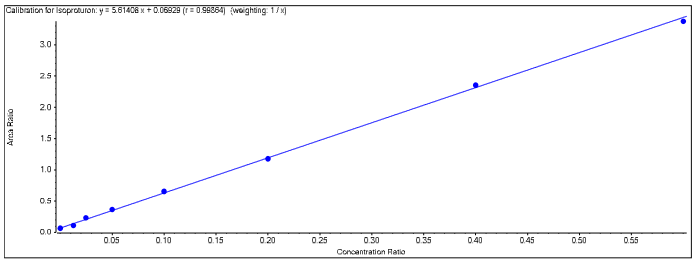

Figure 3. Calibration for Isoproturon from 12.5 – 600 ng/L

Table 3. Signal-to-noise (S/N)* of the lowest calibration standard and r' values taken from calibration lines 12.5 – 600 ng/L

Pesticide	S/N at 12.5 ng/L	'r' value
MCPA	86.7	0.99967
Clopyralid[#]	25.1	0.99769
2,4-D	51.3	0.99963
Dicamba	25.5	0.99856
2,4-DB	25.8	0.99936
Dichlorprop	76.5	0.99934
Bromoxynil	50.3	0.99956
Ioxynil	148.5	0.99932
Bentazone	368.1	0.99888
MCPB	15.3	0.99868
MCPP	102.1	0.99968
Triclopyr	27.6	0.99871
Fluroxypyr	22.3	0.99846
Benazolin	26	0.99876
Aminopyralid	100.7	0.99955
Diuron	41.2	0.99816
Isoproturon	39.5	0.99864
Monolinuron	32	0.99904
Metoxuron	54.9	0.99882
Fenuron	53.1	0.99913
Pencycuron	167.9	0.99982
Linuron	26.2	0.9993
Chlorotoluron	50.5	0.99921

* S/N was calculated in MultiQuant™ software version 2.0.1

[#] S/N of Chlopyralid at 25 ng/L

It can also be seen that every compound with the exception of Clopyralid gave a good signal-to-noise (> 15:1) from the lowest standard 12.5 ng/L (Table 3). Clopyralid, the least sensitive of all the compounds, gave a signal to noise of 25:1 at its lowest standard level of 25 ng/L. There was no carryover observed for either method.

This method has been validated and used routinely for testing water samples as part of surveillance exercises. Normally such tests produce negative results but in certain instances positive results can be observed which normally result from the illegal disposure of pesticides.

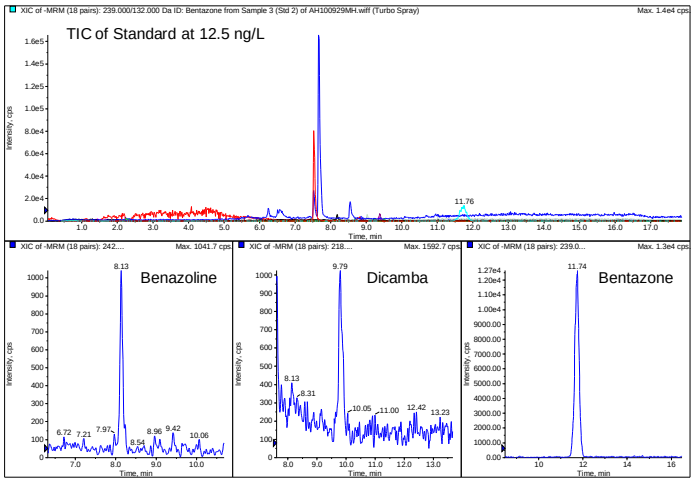

Figure 4a. 12.5 ng/L standard in negative polarity

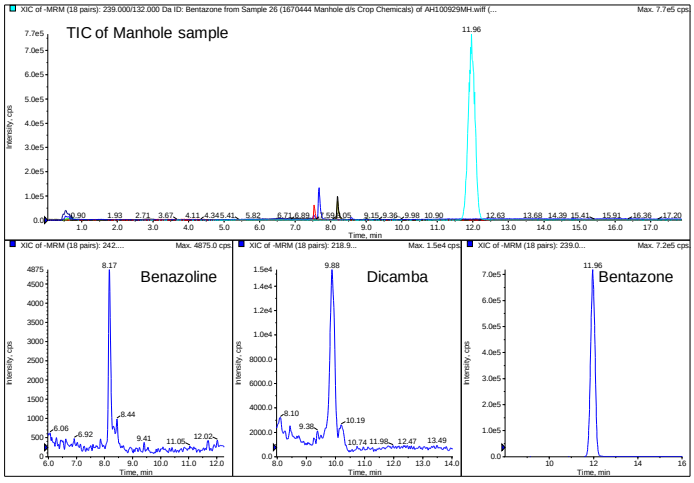

Figure 4b. Manhole sample in negative polarity

Figures 4 and 5 show examples of where this method has detected both the presence of certain acidic herbicides and phenyl ureas in samples of water from manholes. In each example the amount of pesticide detected varies with analyte and is in the parts per trillion range but exceeds the lowest calibration standard.

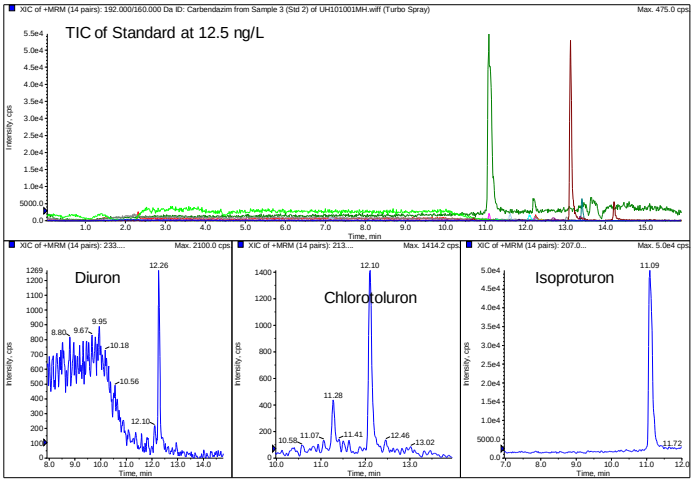

Figure 5a. 12.5 ng/L standard in positive polarity

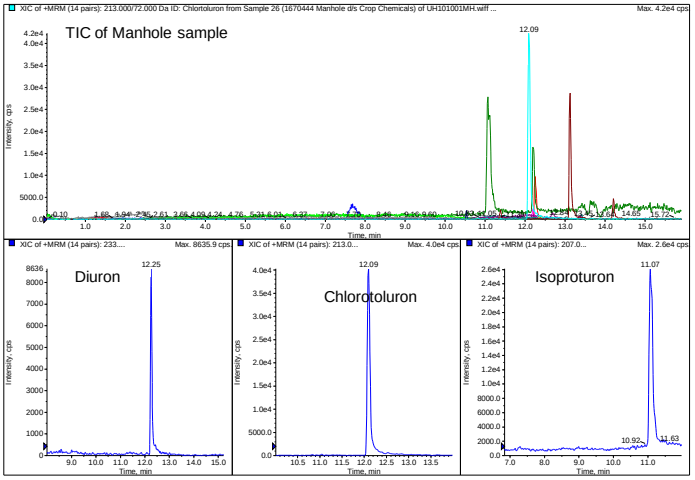

Figure 5b. Manhole sample in positive polarity

Conclusion

The results show that both acidic herbicides and phenyl ureas can be detected at the required limits set by the water industry in the UK. The sample preparation used involved a simple filtration step which removed the cost and time associated with solid phase extraction and/or liquid liquid extraction traditionally used for GC-MS analysis. Acidic herbicides and phenyl urea pesticides ionise under different polarities and require different HPLC conditions to obtain their best sensitivity. Using conventional LC and a timed switching valve samples can be run under the optimised LC conditions for either class of compounds, without supervision. The automated column switching enables researchers to optimise the pH of the mobile phase and column chemistry to produce the best sensitivity for both compound classes.

Such a method has been shown to be robust and sensitive enough to be applied to surveillance work, in the UK, needed to maintain a safe water supply.

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