Application
Note: 391

Analysis of Regulated Pesticides in Drinking Water Using Accela and EQuan

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Key Words

- TSQ Quantum™
- Accela™ LC System
- EQuan™
- LC-MS/MS
- Pesticide Analysis
- Water Analysis

Introduction

Pesticides are used throughout the world to control pests that are harmful to crops, animals, or people. Because of the danger of pesticides to human health and the environment, regulatory agencies control their use and set pesticide residue tolerance levels. The limits of detection (LODs) for many of these substances are at the parts-per-trillion (ppt) level. In order to achieve this level of detection, offline sample pre-concentration is often performed. However, these sample preparation procedures can be time consuming, adding as much as one to two days to the total analysis time. Therefore, a method for online sample pre-concentration that bypasses the offline sample pre-concentration provides a significant time savings over conventional methods.

We describe a method for online sample cleanup and analysis using the EQuan system. This method couples a Fast-HPLC system with two Hypersil™ GOLD LC columns (Thermo Scientific, Bellefonte, PA)–one for pre-concentration of the sample, the second for the analytical separation–and an LC-MS/MS instrument. Large volumes of drinking water samples (1 mL) can be directly injected onto the loading column for LC-MS/MS analysis, thus eliminating the need for offline sample pre-concentration and saving overall analysis time. Using this configuration, run times of six minutes are achieved for the analysis of a mixture of pesticides. For separation prior to analysis using an LC-MS/MS instrument, Fast-HPLC allows for significantly shorter run times than conventional HPLC.

Goal

To demonstrate the use of Fast-HPLC and a large volume injection to analyze sub-ppb concentrations of regulated pesticides in drinking water samples.

Experimental Conditions

Sample Preparation

Bottled drinking water was spiked with a mixture of the following pesticides: carbofuran, carbaryl, diuron, daimuron, bensulfuron-methyl, tricyclazole, azoxystrobin, halosulfuron-methyl, flazasulfuron, thiodicarb, and siduron. Concentrations were prepared at the following levels: 0.5, 1, 5, 10, 50, 100, 500, and 1000 pg/mL (ppt). No other sample treatment was performed prior to injection. The mass transitions and collision energies for each compound are listed in Table 1.

HPLC

Fast-HPLC analysis was performed using the Accela High Speed LC System (Thermo Scientific, San Jose, CA). A 1 mL water sample was injected directly onto a 20 mm×2.1 mm ID, 12 µm Hypersil GOLD loading column in a high aqueous mobile phase at a flow rate of 1 mL/min (see Figure 1a). After approximately one minute, a 6-port valve on the mass spectrometer was switched via the instrument control software. This enabled the loading column to be back flushed onto the analytical column (Hypersil GOLD 50×2.1 mm ID, 1.9 µm), where the compounds were separated prior to introduction into the mass spectrometer (Figure 1b). After all of the compounds were eluted from the analytical column at a

Analyte	Precursor Mass *(m/z)*	Product Mass *(m/z)*	Collision Energy (eV)
Tricyclazole	190.09	106	10
Thiodicarb	355.06	88	14
Carbofuran	355.06	165	14
Carbaryl	202.14	145	10
Diuron	233.05	72	20
Bensulfuron-methyl	411.13	149	22
Flazasulfuron	408.08	182	24
Siduron	233.19	137	20
Azoxystrobin	404.16	372	15
Halosulfuron-methyl	435.11	182	24
Daimuron	269.21	151	14

Table 1: List of mass transitions and collision energies for each compound analyzed.

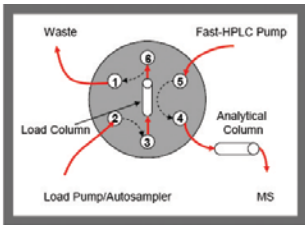


Figure 1a: 6-port valve position one (load position), for loading the sample onto the loading column.

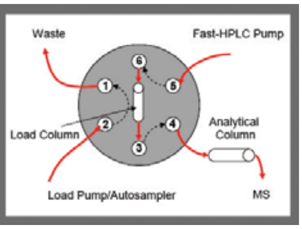


Figure 1b: 6-port valve position two (inject position), for eluting the compounds trapped on the loading column onto the analytical column.

flow rate of 850 µL/min, the 6-port valve was switched back to the starting position. The loading and analytical columns were cleaned with a high organic phase before being re-equilibrated to their starting conditions. The total run time for each analysis was six minutes. The mobile phases for the analysis were water and acetonitrile, both containing 0.1% formic acid. The gradient profile for each pump is shown in Figure 2.

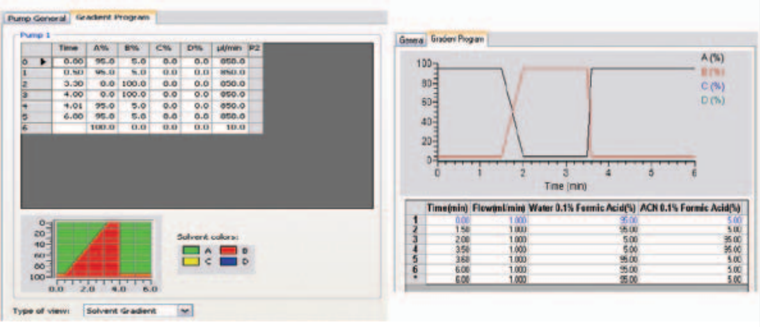
The pressure at the beginning of the gradient was monitored. At a flow rate of 850 µL/min (at the initial gradient conditions with the flow going through only the Hypersil GOLD 50×2.1 mm, 1.9 µm column), the backpressure for the Fast-HPLC system was approximately 450 bar. For comparison, an earlier method which used a Hypersil GOLD 50×2.1 mm, 3 µm column had a backpressure of approximately 150 bar at a flow rate of 200 µL/min.

MS

MS analysis was carried out on a TSQ Quantum Access triple stage quadrupole mass spectrometer with a heated electrospray ionization (H-ESI) probe (Thermo Scientific, San Jose, CA). The MS conditions were as follows:
Ion source polarity: Positive ion mode
Spray voltage: 4000 V
Vaporizer temperature: 450 °C
Sheath gas pressure (N_2): 50 units
Auxiliary gas pressure (N_2): 50 units
Ion transfer tube temperature: 380 °C
Collision Gas (Ar): 1.0 mTorr
Q1/Q3 Peak Resolution: 0.7 u
Scan Width: 0.002 u

Results and Discussion

Chromatograms for the calibration standard at a concentration of 500 pg/mL are shown in Figure 3. In the Fast-HPLC run, all 11 of the individual analytes were eluted before three minutes. In contrast, none of the analytes in the standard HPLC run were eluted until nearly eight minutes into the run. Further optimization of the chromatography for the Fast-HPLC would produce even shorter run times.



	Time	A%	B%	C%	D%	µl/min	P2
0 ▶	0.00	95.0	5.0	0.0	0.0	850.0	
1	0.50	95.0	5.0	0.0	0.0	850.0	
2	3.30	0.0	100.0	0.0	0.0	850.0	
3	4.00	0.0	100.0	0.0	0.0	850.0	
4	4.01	95.0	5.0	0.0	0.0	850.0	
5	6.00	95.0	5.0	0.0	0.0	850.0	
6		100.0	0.0	0.0	0.0	10.0	

	Time(min)	Flow(ml/min)	Water 0.1% Formic Acid(%)	ACN 0.1% Formic Acid(%)
1	0.00	1.000	95.00	5.00
2	1.50	1.000	95.00	5.00
3	2.00	1.000	5.00	95.00
4	3.50	1.000	5.00	95.00
5	3.60	1.000	95.00	5.00
6	6.00	1.000	95.00	5.00
*	6.00	1.000	95.00	5.00

Figure 2: Gradient profiles for the two LC pumps used in this experiment. The Fast-HPLC pump gradient is shown on the left, and the loading pump gradient is show on the right.

Calibration curves for all 11 compounds were generated using LCQUAN™ 2.5 software (Thermo Scientific, San Jose, CA). Excellent linearity was achieved for all of the compounds analyzed in this experiment. Figure 4 shows a representative calibration curve for the compound azoxystrobin over the concentration range 0.5 to 1000 pg/mL (ppt). The calibration curve fit parameters and the limits of detection for the analytes are summarized in Table 2. The final column in the table lists the Minimum Performance Reporting Limit (MPRL) for these compounds as set by the Japanese Ministry of Health, Labour, and Welfare[1]. All of the compounds were detected and quantified at levels well below these regulatory requirements.

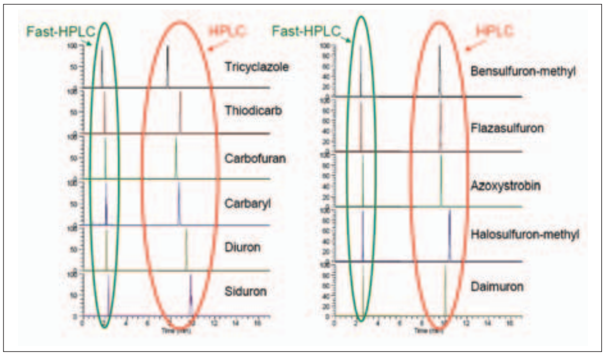


Figure 3: Chromatograms showing the SRMs for each of the components in the mixture. Two different HPLC conditions are shown: the Fast-HPLC run and the standard HPLC run. All compounds in the Fast-HPLC run are eluted in less than three minutes (circled in green). Those in the standard HPLC run are eluted much later (circled in red). These chromatograms represent a calibration level of 500 pg/mL (ppt).

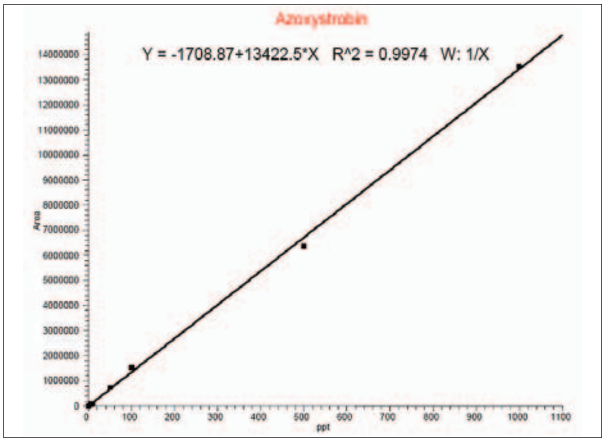


Figure 4: Calibration curve for the compound azoxystrobin. This calibration curve covers the range from 0.5 to 1000 pg/mL (ppt)

Analyte	R^2	Limit of Detection (ppt)	MPRL (ppt)
Tricyclazole	0.9972	0.5	800
Thiodicarb	0.9930	5	800
Carbofuran	0.9928	1	50
Carbaryl	0.9345	100	500
Diuron	0.9978	100	200
Bensulfuron-methyl	0.9933	0.5	4000
Flazasulfuron	0.9944	1	300
Siduron	0.9973	0.5	3000
Azoxystrobin	0.9974	0.5	5000

Table 2: List of calibration curve fit parameters, limits of detection, and Minimum Performance Reporting Levels (MPRL) for each compound from the Japanese Ministry of Health, Labour and Welfare. All calibrations were carried our using a linear curve fit and a weighting factor of 1/X.

Conclusion

The implementation of Fast-HPLC, coupled with the online pre-concentration and sample preparation technique EQuan, yielded analysis of 11 pesticides in drinking water in less than one-third the time of conventional HPLC analysis. All of the compounds eluted within three minutes, which included a one-minute loading time for the sample to be pre-concentrated on the loading column. The total run time for the analysis was six minutes. The Fast-HPLC method can be further shortened to produce faster chromatographic run times.

The use of large volume injections achieved results below the MPRL regulatory requirements for each of the 11 pesticides. Because the limits of detection were much lower than the MPRL values, the integrated peaks yielded excellent signal-to-noise ratios and allowed for confidence in reporting the results.

Reference

[1] http://www.mhlw.go.jp/index.html (Japanese language version), http://www.mhlw.go.jp/english/index.html (English language version)

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