APPLICATION NOTE

Liquid Chromatography

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Rapid UHPLC Determination of Nine Common Herbicides in Drinking Water with the PerkinElmer Flexar FX-15 System Equipped with a PDA Detector

Introduction

Water supports the physiological activities of any biological cell and is therefore essential for life and the biodiversity of our planet. There are three main sources of water: ocean water, surface water and ground water; among them, ground water stored in aquifers makes up ninety percent of drinkable water in the world and is the largest reservoir of fresh water on the planet. However, ground water is susceptible to pollution by herbicides widely used in agriculture to control unwanted plants. Water polluted by herbicides leach and runoff can cause human health problems including cancer tumors, reproduction deformity, disruption of the endocrine system and DNA damage.

In Karakalpakia (Aral Sea region in central Asia), the United Nations Environmental Protection Program (UNEP) attributed the increase in cancer mortality by 200% and the increase of newborn deformities back in the 1980's to drinking water that was contaminated by pesticides. Around the world constant monitoring is crucial for detecting harmful levels of herbicides in drinking water.

In the U.S., the Clean Water Act (CWA) regulates the discharge of pollutants into surface water. Furthermore the Environmental Protection Agency (EPA) has established limits for any pollutant in drinking water. It is however in the best interest of local municipalities as well as the food industries to routinely test water to ensure its safety and compliance to regulations.

This application presents a sensitive and robust liquid chromatography method to test nine widely used herbicides (Figure 1), using a 3 µm UHPLC column to achieve very high throughput to reduce testing time and solvent consumption. The throughput is compared to that of a conventional C18 HPLC column. Method conditions and performance data including precision and linearity, are presented.

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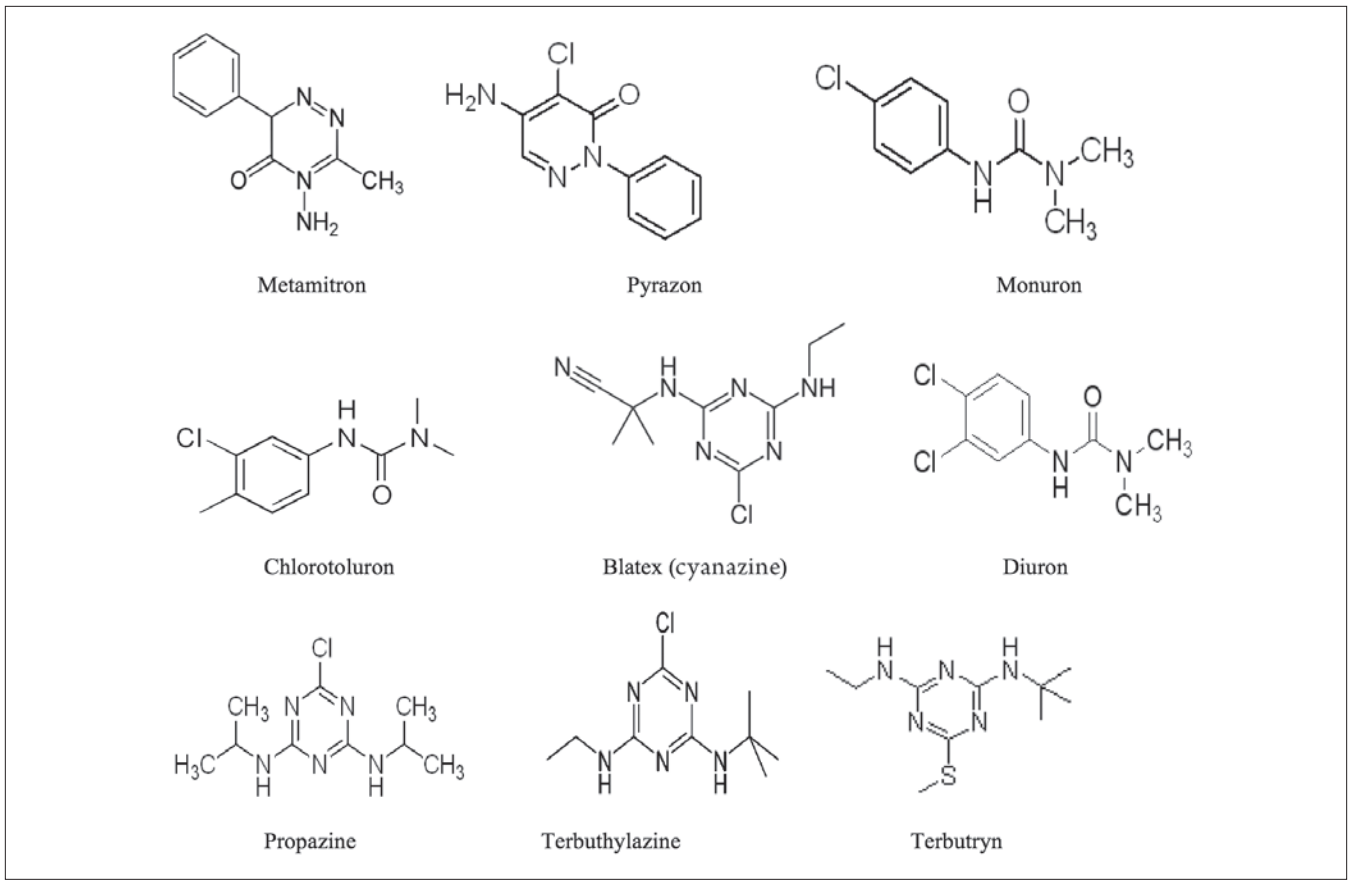

Figure 1. Names and structure of nine herbicides studied.

Experimental

A working solution containing 20-28 µg/mL of each herbicide was prepared by dilution from neat material using water as a solvent. Precision was evaluated with six injections of the working standard. Linearity was determined across the range of 125-28000 ng/mL. A sample of purified water was spiked with the working standard to obtain a solution between 0.5-0.7 µg/mL.

A PerkinElmer® Flexar™ FX-15 UHPLC system fitted with a Flexar FX PDA photodiode array detector was used. The separation was achieved using a Restek® Pinnacle® DB C18, 3 µm, 100 x 2.1 mm. The run time was approximately eight minutes with a back pressure of 8500 PSI (586 bar).

***Table 1.* Detailed UHPLC system and chromatographic conditions.**

Autosampler:	Flexar FX UHPLC Setting: 50 µL loop and 15 µL needle volume, partial loop mode Injection: 5 µL; injector wash and carrier:water
Detector:	Flexar FX PDA UHPLC Detector
Analytical Wavelength:	225 nm
Pump:	Flexar FX-15
Column:	Xterra® MS C18, 3.5 µm, 100 x 4.6 mm Restek® Pinnacle® DB C18, 3 µm, 100 x 2.1 mm (Cat #9414312)
Mobile phase:	B: acetonitrile, A: water (HPLC grade solvent)
Software:	Chromera® Version 3.0

Conventional LC Column at 30 °C

Time (min)	Flow rate (mL/min)	B %	Curve
16	1.0	10-45	1

UHPLC Column at 50 °C

Time (min)	Flow rate (mL/min)	B %	Curve
8	1.0	15-45	1

Two min. equilibration after injection.

Results And Discussion

Initially, the method was developed with a conventional C18 LC column. The optimal flow rate of this method was determined to be 1.0 mL/min. at a temperature of 30 °C. All the herbicide peaks eluted within 16 min. (Figure 2). Eight minutes run time was achieved by using a column design for UHPLC that is capable of sustaining a pressure up to 15000 Psi and a temperature up to 80 °C. The selectivity, capacity and resolution were significantly improved (Figures 3 and 4).

In addition to the reduction of the run time and the solvent usage by half, the resolutions of analyte peaks were improved. The optimal flow rate was 1.0 mL/min. at a temperature of 50 °C and an improved separation was obtained.

The final analysis was completed in eight min. with a total solvent usage of 8.0 mL per injection, an impressive improvement from 16 min. run time and 16.0 mL solvent usage when the conventional HPLC column was used. This is important not only because of the relatively high cost of HPLC-grade solvents, but also because far less solvent must be disposed of as waste. This results in much lower cost of ownership and a much "greener" laboratory operation.

Overall, excellent method performance was achieved. The linearity of the analysis achieved an average r^2 value of 1. The average precision was 1.1% ranging from 0.6-1.4% RSD. The average recovery for spiked sample was 102% ranging from 97-109%. Details of the method performance are presented in Table 2.

A spectrum of each herbicide was obtained from the analysis of the standard solution over a range of 190 nm to 700 nm, and the wavelength maximum was determined, enabling the selection of a suitable wavelength setting for the analysis. A spectral library was created within Chromera using the standard solution run, and was used to confirm the identity of the peaks in spiked water samples.

An annotated UHPLC chromatogram of the spiked water sample and the spectra of five herbicides are shown in Figures 5 and 6.

Confirming the identity of compounds in the chromatogram of a known or an unknown sample is an important aspect of quality assurance, and adds another level of confidence to the analysis. Confirmation of the presence of herbicides in the spiked samples is done by using the spectral library function allowing the comparison of the spectra at the peak apexes to the spectra from the standard solution previously stored in the spectral library.

Table 2. **Precision, linearity and recovery.**

Compound responses	% RSD (n=6) ng/mL*	Linearity range	r^2 recovery %	Spiked water
Metamitron	1.4	175-28000	0.9999	106
Pyrazon	1.4	125-20000	1	103
Monuron	1.0	175-28000	1	97
Blatex	1.2	175-28000	1	102
Chlorotoluron	1.0	125-20000	1	95
Diuron	1.2	175-28000	0.9999	101
Propazine	1.0	125-20000	1	106
Terbuthylazine	1.0	125-20000	0.9999	109
Terbutryn	0.6	125-20000	0.9998	98

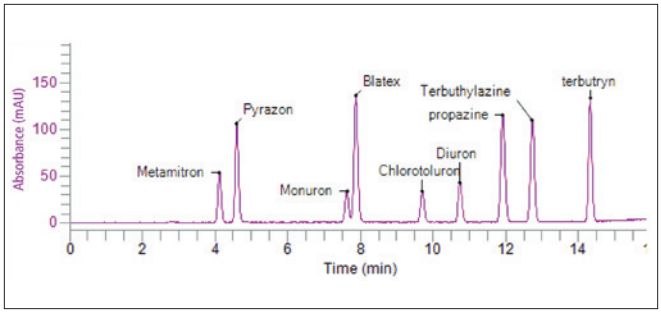

Figure 2. Chromatogram from the analysis of a standard solution of herbicides with a conventional HPLC column.

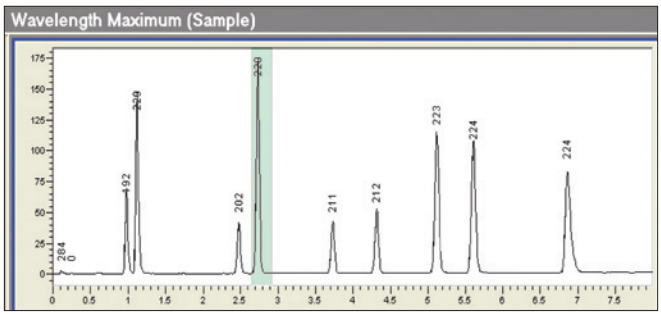

Figure 3. Chromatogram from the analysis of a standard solution of herbicides with a UHPLC column showing maximum wavelength.

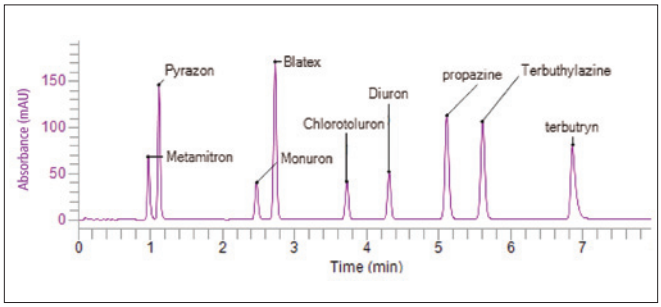

Figure 4. Chromatogram from the analysis of a standard solution of herbicides with a UHPLC column.

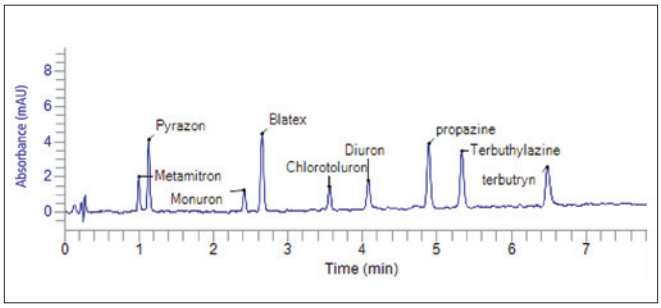

Figure 5. Chromatogram from the analysis of purified water spiked with herbicides.

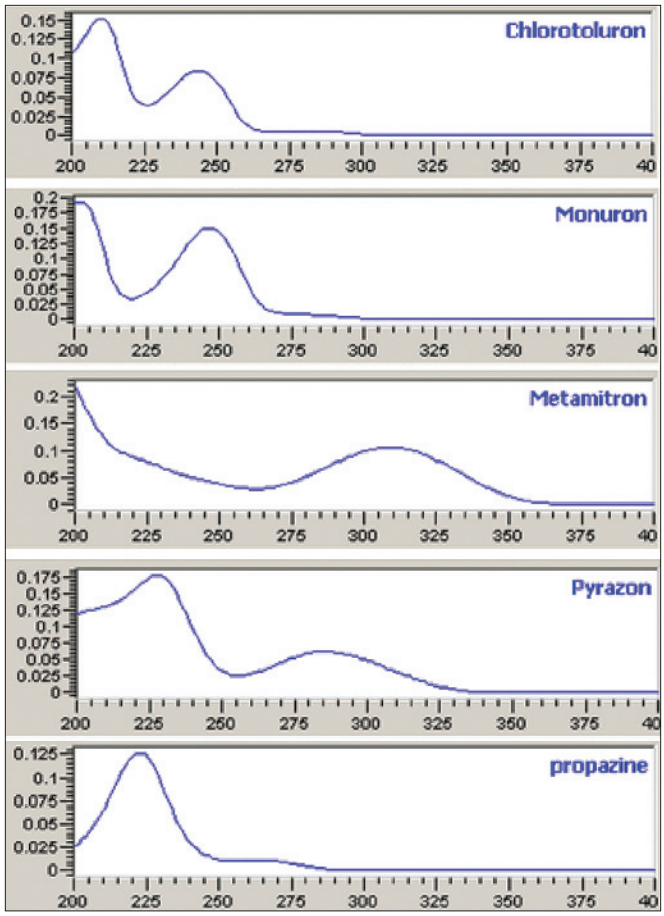

Figure 6. Stored spectra of five herbicides from the analysis of a standard solution.

Conclusion

The application of UHPLC to the analysis of nine herbicides has resulted in about 50% reduction in run time, as well as a reduction of solvent usage by half when compared to the conventional HPLC analysis. The PerkinElmer Flexar FX-15 UHPLC system and Restek® Pinnacle® DB C18, 3 µm, 100 x 2.1 mm column resolved all the nine herbicides studied in about eight minutes and the method was shown to be linear. The PerkinElmer FX PDA provides a rugged and accurate detection over a range of 190 nm to 700 nm, encompassing UV and visible wavelengths. PerkinElmer's Chromera software offers many data acquisition and processing features: spectral library creation, and peak purity, spectra 3D and contour maps, which are powerful tools for interrogating the information content of a 3d photodiode array chromatogram. The spectral library search function allowed the storage of standard herbicides spectra, later used for peaks identification and confirmation in sample.

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6. Clean Water act 33 U.S.C. §1251 et seq. (1972).

7. Toxic Substance Control Act. 15 U.S.C. §2601 et seq. (1976).

8. 40 CFR: Protection of the Environment: Chapter 1, Subchapter E Pesticide Programs (parts 150-189).

Note: This application is subject to change without prior notice.

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