ENVIRONMENTAL ANALYSIS

MONITORING FOR PHARMACEUTICALS IN SURFACE WATER USING DIRECT AQUEOUS INJECTION ON THE AGILENT 6490 LC/QQQ

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Solution Note

Author

Neil Cullum

Anglian Water Services, Huntingdon, UK

Abstract

A method for the determination of a broad suite of pharmaceuticals in surface water has been developed on the Agilent 6490 LC/QQQ. The method utilises direct aqueous injection onto the LC/QQQ and achieves limits of detection of between 2 - 10 ng/L (0.002 - 0.010 µg/L).

Introduction

The ubiquitous use of pharmaceuticals, both prescribed and over the counter, has resulted in a relatively continuous discharge of pharmaceuticals and their metabolites into wastewater. A range of pharmaceuticals, including hormones, antibiotics, NSAIDS, antidepressants and antifungal agents have been detected in surface waters and groundwaters across the world. Whilst concentrations are generally low, sub µg/L levels in surface waters, their presence has raised concerns over their potential to enter drinking water supplies and their potential risk to human health [1, 2].

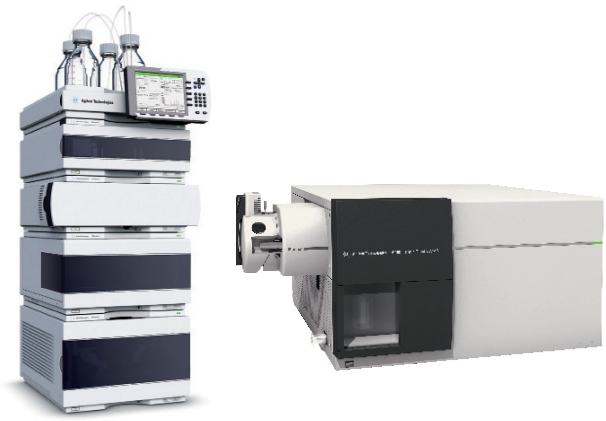
In Europe, the Drinking Water Directive 98/83/EC does not currently propose any guideline values for pharmaceutical compounds in drinking water and no Environmental Quality Standard (EQS) levels have been set for surface waters. However, following a comprehensive consultation and assessment process, the European Commission proposed on the 31st January 2012, to add a further 15 new substances to the priority list of those known to pose a pollution risk to surface waters [3].

The proposed additional substances include pharmaceuticals for the first time (17 alpha-ethinylestradiol, 17 beta-estradiol and Diclofenac). These new rules amend the Water Framework Directive (WFD 2000/60/EC) as well as the EQS Directive (2008/105/EC). Anglian Water has developed a method for the determination of a broad suite of pharmaceuticals in surface water using direct aqueous injection on an Agilent 6490 LC/QQQ (high performance liquid chromatography with mass spectrometer triple quadrupole detection) system. This method can determine a total of 30 compounds using both positive and negative ionisation modes. The positive ion suite comprises 20 compounds, while the negative ion suite 10 compounds. The method benefits from requiring no sample preparation but is sensitive enough to achieve limits of detection (LODs) of between 2 – 10 ng/L for all compounds. The range of application for all compounds is up to 250 ng/L.

ANALYTICAL TECHNIQUE

Sample Preparation

- Direct aqueous injection onto the Agilent 6490 LC/QQQ
- Positive Ion Mode: 20 Compounds, 6 Internal Standards, 25 µL injection.
- Negative Ion Mode: 10 Compounds, 5 Internal Standards, 100 µL injection.



Instrumentation

Agilent 1200 Series HPLC System consisting of the following:

- Autosampler G1329C
- Micro Vacuum Degasser G1379B
- 2 Position / 6 Port Valve G4231A
- Binary Pump G1312B
- Column Compartment G1316A

Agilent 6490 QQQ Ion Funnel System

Agilent Column: Zorbax Eclipse Plus C18 2.1 x 150mm x 3.5µm

POSITIVE ION MODE				NEGATIVE ION MODE	
Compound	**Class**	**Compound**	**Class**	**Compound**	**Class**
Ofloxacin	Antibiotic	Cyclophosphamide	Cancer Treatment	Ibuprofen	NSAID
Oxytetracycline	Antibiotic	Diphenhydramine	Anti-Histamine	Triclosan	Antibacterial
Erythromycin	Antibiotic	Nor-Fluoxetine	Metabolite	Diclofenac	NSAID
Propranolol	Beta-Blocker	Ranitidine	Ulcer Treatment	Atorvastatin	Statin
Fluoxetine	Antidepressant	Simvastatin	Statin	Chloramphenicol	Antibiotic
ASMX	Metabolite	Sulpamethoxazole	Antibiotic	Clofibric Acid	Metabolite
Atenolol	Beta-Blocker	Tramadol	Opioid Analgesic	Furosemide	Diuretic
Carbamazepine	Anti-Epileptic	Trimethoprim	Antibiotic	Gemfibrozil	Lipid Lowering
Carbamazepine Epoxide	Metabolite	Orlistat	Obesity Treatment	Ketoprofen	NSAID
Citalopram	Antidepressant	Tamoxifen	Cancer Treatment	Naproxen	NSAID

Results and Discussion

An Agilent 6490 Ion Funnel LC/QQQ System, with an Agilent Zorbax Eclipse column, was used to determine the pharmaceutical compounds. A method was developed that was able to determine all 30 compounds, with 20 compounds being determined in positive ionisation mode and 10 in negative ionisation mode. Standards and samples were introduced onto the system by direct aqueous injection, eliminating the need for costly and time consuming sample preparation techniques. Smaller sample volumes are required to be taken and results can be obtained faster. The method should also be more reproducible as there are no extraction variables.

Using this method, recoveries of the pharmaceutical compounds ranged from 86.6 – 120.6 %, whilst limits of detection were compound dependent and ranged from 2 – 10 ng/L (0.002 – 0.010 µg/L).

Positive Ionisation Suite

The calibration range for each compound in the positive ion suite was 0 to 250 ng/L, with standards at 0, 50, 100, 150 and 250 ng/L. Figure 1 shows a typical chromatogram of a 50 ng/L standard. The run time on this suite is 23 minutes. Good chromatography has been achieved for all compounds. The results of a river water sample analysed on this suite are shown in Table 1. Out of the 20 compounds in this suite, 12 were found, with concentrations ranging from 2ng/L to 123ng/L. Figure 2 shows the calibration curve and compound information for Propranolol (18ng/L) and Figure 3 the calibration curve and compound information for Tramadol (124 ng/L). A total of 431 ng/L of pharmaceuticals were detected.

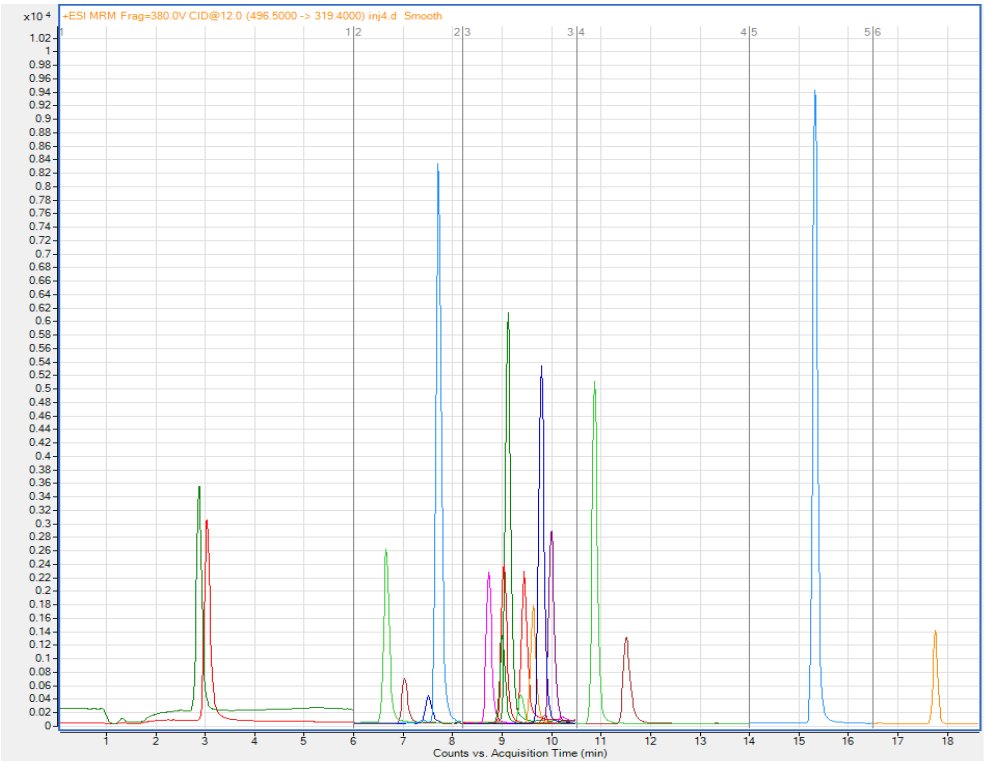


Figure 1. 50 ng/L Standard Chromatogram in Positive Ionisation Mode.

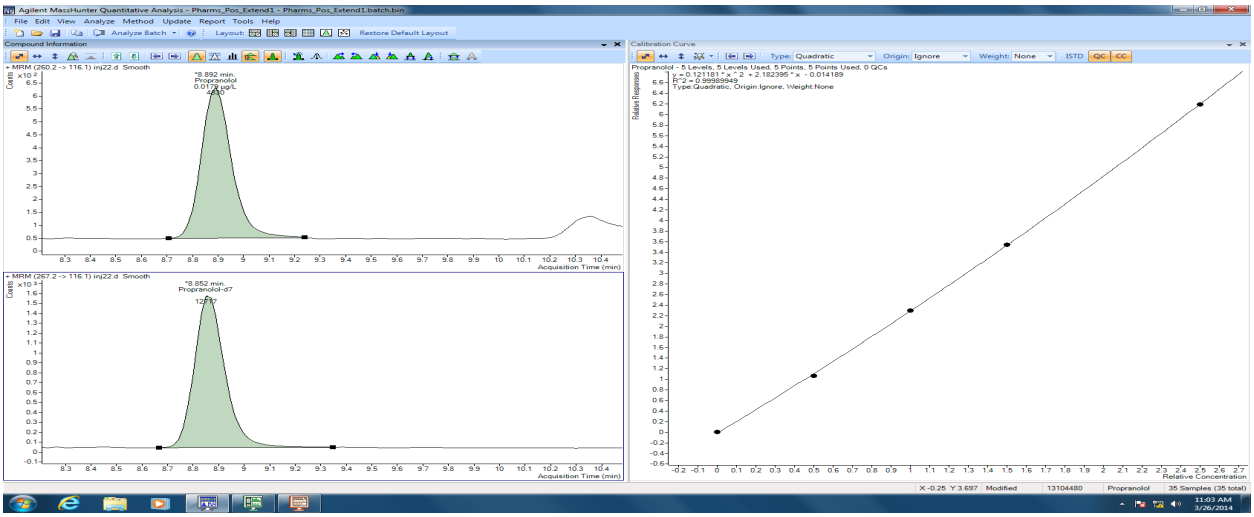


Figure 2. Propranolol Compound Information and Calibration Curve.

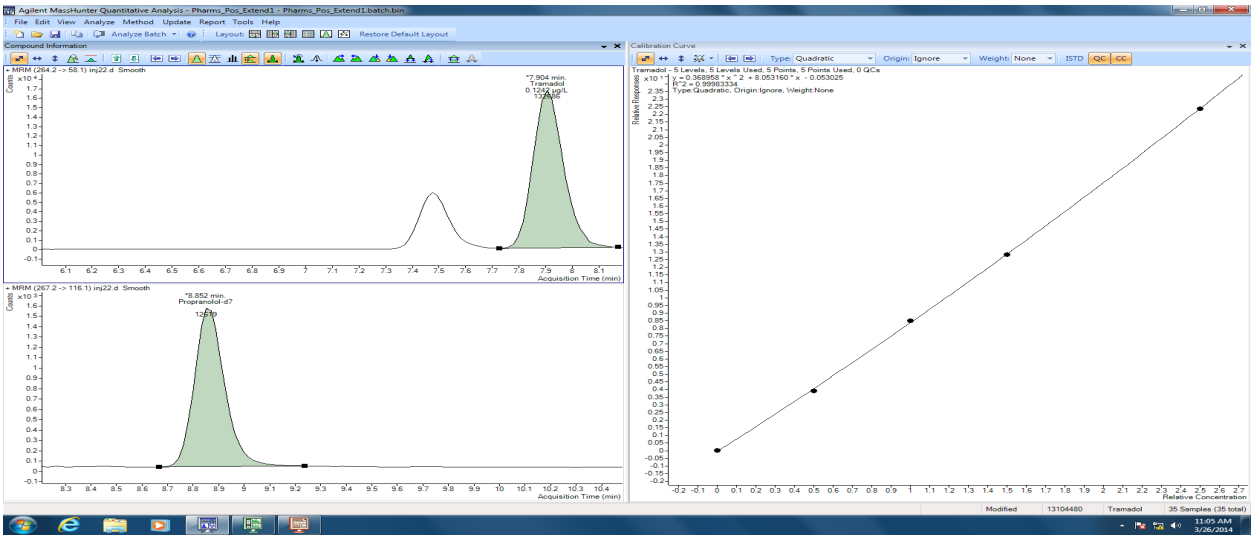


Figure 3. Tramadol Compound Information and Calibration Curve.

Compound	Concentration, µg/L	Compound	Concentration, µg/L
Ofloxacin	<0.002	Cyclophosphamide	<0.002
Oxytetracycline	0.003	Diphenhydramine	<0.002
Erythromycin	0.066	Nor-Fluoxetine	<0.002
Propranolol	0.018	Ranitidine	0.042
Fluoxetine	<0.002	Simvastatin	<0.002
ASMX	0.002	Sulfamethoxazole	0.014
Atenolol	0.039	Tramadol	0.124
Carbamazepine	0.055	Trimethoprim	0.041
Carbamazepine Epoxide	0.010	Orlistat	<0.005
Citalopram	0.017	Tamoxifen	<0.002

Table 1. Results for a River Water sample in Positive Ionisation Mode.

Negative Ionisation Suite

The calibration range for each compound in the negative ion suite was 0 to 250 ng/L, with standards at 0, 50, 100, 150 and 250 ng/L. Figure 4 shows a typical chromatogram of a 100 ng/L standard. The run time on this suite is 21 minutes. Good chromatography has been achieved for all compounds. The results of a river water sample analysed on this suite are shown in Table 2. Out of the 10 compounds in this suite, 6 were found, with concentrations ranging from 5 ng/L to 81 ng/L. Figure 5 shows the calibration curve and compound information for Diclofenac (33 ng/L) and Figure 6 the calibration curve and compound information for Naproxen (80 ng/L). A total of 197 ng/L of pharmaceuticals were detected.

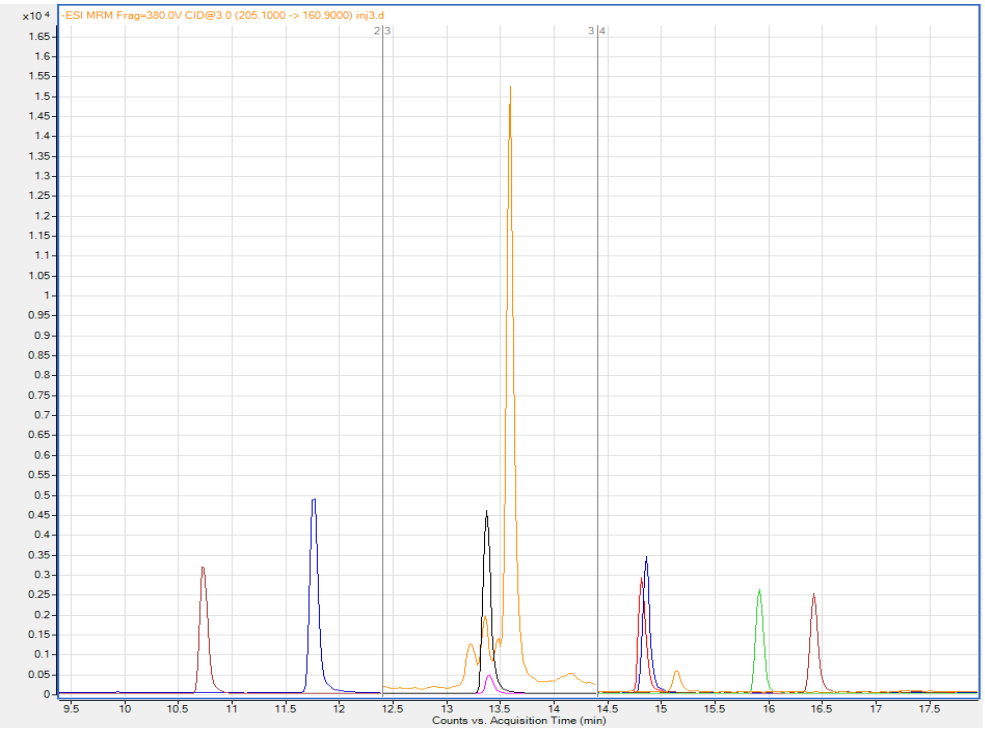


Figure 4. 100 ng/L Standard Chromatogram in Negative Ionisation Mode.

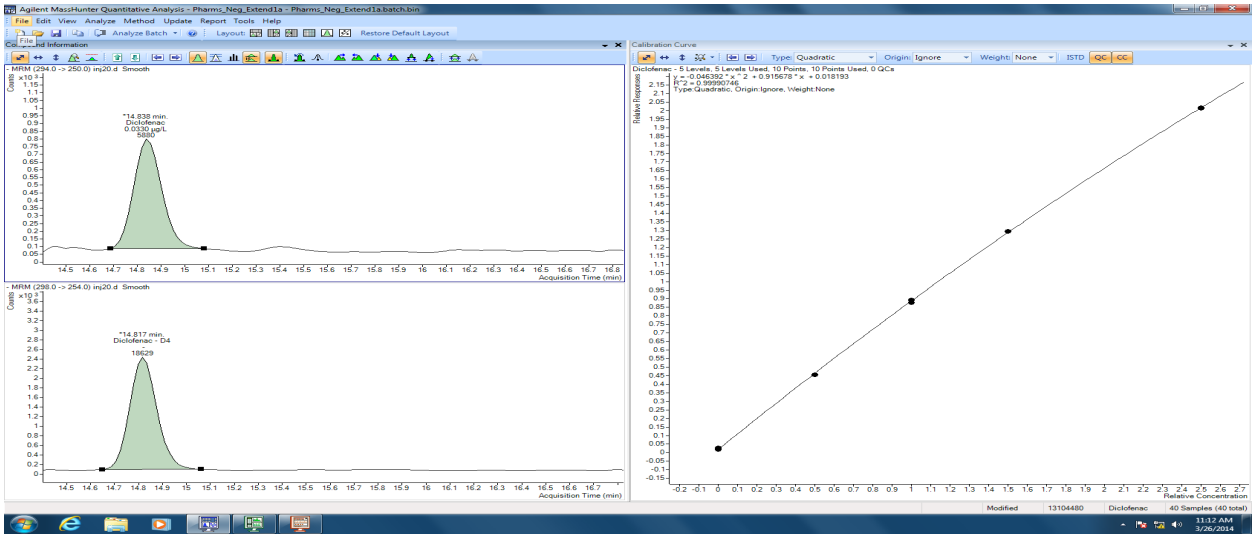


Figure 5. Diclofenac Compound Information and Calibration Curve.

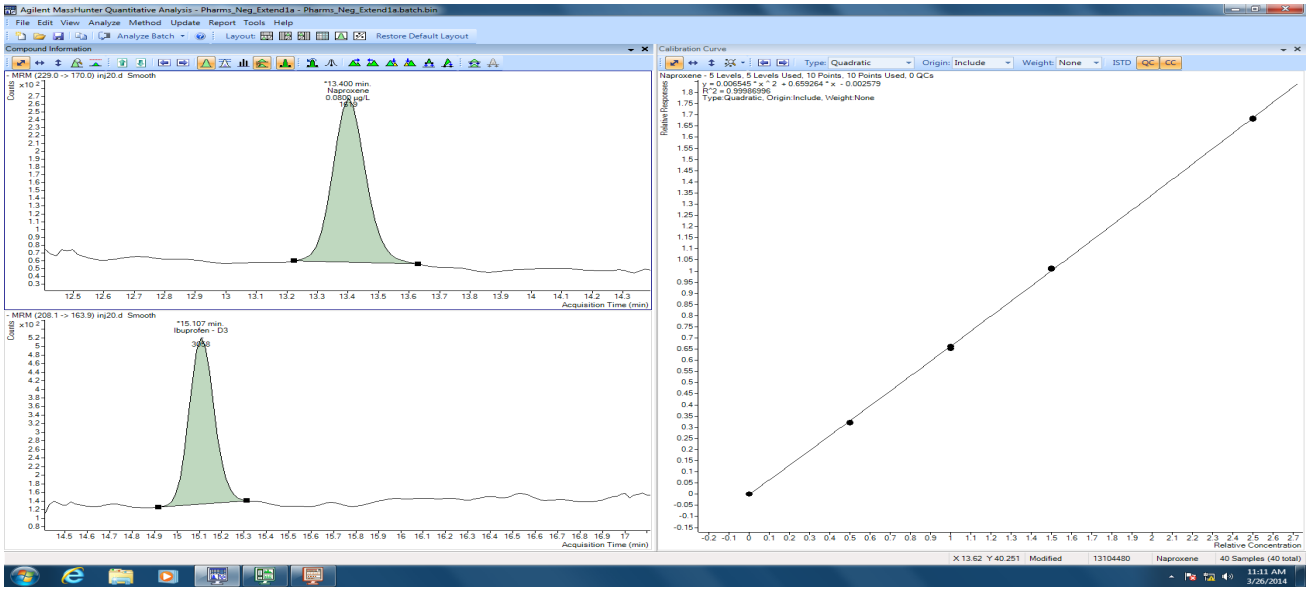


Figure 6. Naproxen Compound Information and Calibration Curve.

Compound	Concentration, µg/L	Compound	Concentration, µg/L
Ibuprofen	0.032	Clofibric Acid	<0.002
Triclosan	0.008	Furosemide	0.039
Dichlofenac	0.033	Gemfibrozil	<0.002
Atorvastatin	0.005	Ketoprofen	<0.005
Chloramphenicol	<0.002	Naproxen	0.080

Table 2. Results for a River Water sample in Negative Ionisation Mode.

Conclusions

A method has been developed for the determination of a broad suite of pharmaceuticals in surface water using direct aqueous injection on the Agilent 6490 LC/QQQ. This method demonstrates excellent sensitivity and is capable of achieving LODs of between 2 – 10 ng/L (0.002 – 0.010 µg/L).

References

1. A.B.A. Boxall, S.C. Monteiro, R. Fussell, R.J.Williams, J. Bruemer, R. Greenwood and P. Bersuder, 'Targeted monitoring for human pharmaceuticals in vulnerable source and final waters,' DWI 70/2/231, December 2011.
2. World Health Organization, 'Pharmaceuticals in drinking water,' WHO/HSE/WSH/11.05, 2011.
3. European Commission, Brussels, 31.1.2012, 'Proposal for a Directive of the European Parliament and of the Council amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy,' COM(2011) 876 final.

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Published in USA, April 10, 2014
5991-4394ENE

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