Analysis of Pharmaceuticals in Water Using Automated On-line SPE-LC-MS/MS

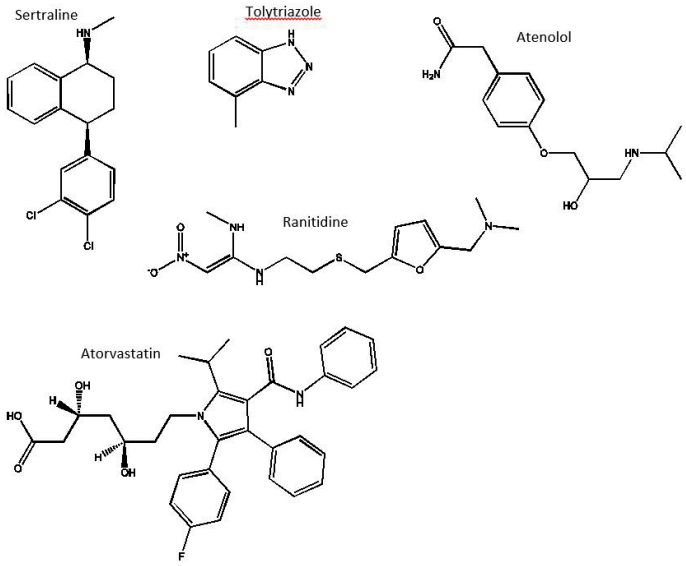

Figure 1. Molecular structures of selected analytes

Introduction

The analytical challenge of measuring emerging contaminants in the environment has been a major research focus of scientists for the last 20 years. Pharmaceuticals and personal care products are an important group of contaminants that have been targeted. Contaminants are usually present in the environmental samples at very low concentration levels (ng L^{-1}) and, for this reason, solid phase extraction techniques are often used to isolate and pre-concentrate the organic compounds of interest.

The on-line approach to sample preparation has grown in popularity because of its advantages in improved workflow and reduced sample handling:

- Little or no sample pre-treatment
- Totally automated procedures
- High precision and accuracy
- Elimination of blow down and reconstitution steps
- Reduced solvent use and disposal costs

Trace analysis of organics in water traditionally involves large sample volumes, labor intensive procedures and relatively high use of solvents. The on-line SPE approach uses a simple, well established hardware setup, and fully integrates sample preparation into the analytical workflow. Typical sample volumes of 1–10 mL, and lower solvent usage mean sample collection, transport and handling, along with solvent disposal costs, are much reduced.

This application note describes the use of EVOLUTE® EXPRESS ABN on-line cartridges in a fully automated on-line SPE-LC-MS/MS method for extraction and quantification of 13 commonly prescribed pharmaceuticals (with a range of characteristics) in water.

Analytes

Atenolol, Ranitidine, Ciprofloxacin, Tolytriazole, Azithomycin, Erythromycin, Prednisolone, Carbamazepine, Clarithromycin, Fluoxetine, Sertraline, Atorvastatin, Diclofenac

Sample Preparation Procedure

Format:

EVOLUTE® EXPRESS On-line cartridge 30 mm x 2.1 mm, part number OSPE-620-32150

Overview

1 mL of spiked sample was injected and loaded (trapped) onto the EVOLUTE® EXPRESS ABN on-line SPE cartridge using a mobile phase consisting of 2% acetonitrile (aq). After 4 mins, valve positions were switched to enable transfer of the analytes (in backflush mode) onto the analytical column using 20% acetonitrile/aq formic acid. Analytes were separated using the gradient conditions shown overleaf, while the SPE column was re-equilibrated prior to the next sample injection. The total cycle time was 20 mins, and a schematic of the process is shown in figure 2.

Experimental

Pharmaceuticals were spiked into representative hard water samples (Ballygowan® Still Natural Mineral Water) at a concentration of 75 ng L^{-1}.

Matrix-matched external standards were prepared from a different stock solution in Ballygowan water at the following concentrations: 0, 25, 50, 75, 100 and 150 ng L^{-1}. Standards were injected in duplicate from high to low concentration. MRM peak areas were used to construct calibration curves and determine the resulting regression coefficient (r^2) using MassLynx 4.0 (Waters). Limit of detection (LOD) was estimated using Excel to be 4x *SD* of the blank (n=4).

Recovery for each analyte was calculated in Excel as a mean of the interpolated peak area (n=4) of the spike on the appropriate calibration line. Method repeatability was estimated in Excel using replicate RSD (n=4) for each analyte.

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On-Line Sample Preparation

System: Autosampler
PAL-RTC

10-port loading valve

6-port selection valve

10 mL sample syringe

Loading Pump
Shimadzu LC-20AD with LPGE/DGU-20A5R

Elution Pump
Shimadzu LC-20AD with LPGE/DGU-20A5R

Loading/Separation Methods
LC-20AD front panel

Acquisition Control / Data System
Waters MassLynx 4.0

MS-MS
Waters Ultima Pt QQQ

System Dwell Volume
1.9 mL

SPE Conditions

Injection
1 mL full loop, 1.25x loop overfill

Loading Flow
2 mL min^{-1}

SPE Loading Conditions
2% acetonitrile (aq)

Loading Duration
4.0 min

HPLC Conditions

HPLC Column
Thames RESTEK Raptor ARC18 2.7 µm, 100 mm x 2.1 mm

HPLC Flow
0.4 mL min^{-1}

HPLC Mobile Phases
A: 0.1% formic acid (aq) / B: acetonitrile

HPLC Initial Conditions
0.1% formic acid (aq) : acetonitrile (20:80 v/v)

HPLC Gradient

Time (min)	%B
0.01	20
1.00	20
7.00	60
7.50	95
11.50	95
11.51	100
13.50	100
14.00	20

Table 1. MS/MS Conditions

Target Compound	Precursor m/z	Product m/z	Cone / v	CE / v
Atenolol	267.2	190.2	55	18
Ranitidine	315.3	176.1	35	16
Ciprofloxacin	332.3	288.3	45	16
Tolytriazole	134.2	79.2	35	15
Azithromycin	749.5	591.5	35	30
Erythromycin	734.5	158.2	55	31
Prednisolone	361.3	343.4	35	9
Carbamazepine	237.1	194.1	65	17
Clarithromycin	748.75	158.1	35	28
Fluoxetine	310.2	148.2	45	7
Sertraline	306.3	159.0	35	24
Atorvastatin	557.3	278.3	35	42
Diclofenac	294.1	250.1	40	11

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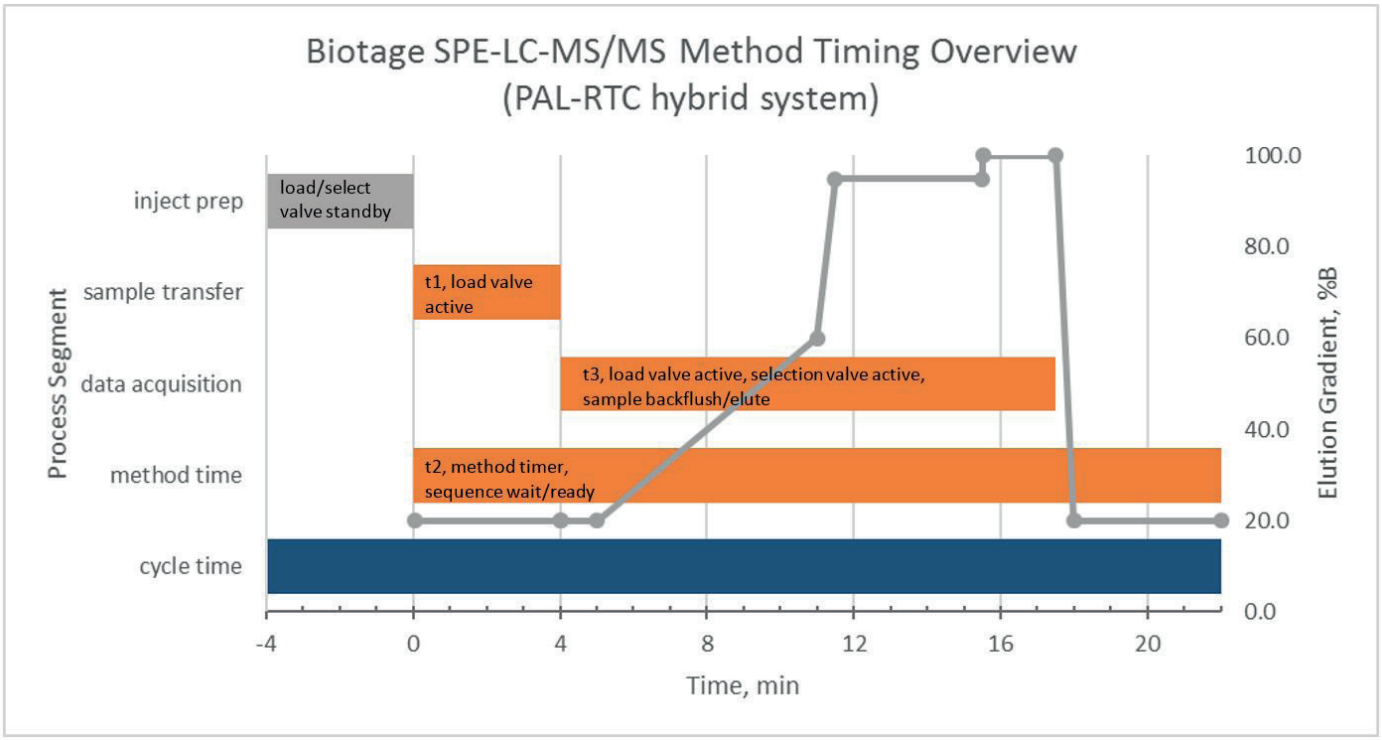

Figure 2. Schematic of the on-line SPE-HPLC process.

Table 2. Results

Target Compound	Mean Recovery % (n=4)	RSD %	Range, ng L^{1}	r^2	LOD, ng L^{-1}
Atenolol	101.2	5.2	0.2–150	0.998	<0.2
Ranitidine	88.2	8.4	1.5–150	0.998	1.5
Ciprofloxacin	100.5	4.9	2.3–150	0.993	2.3
Tolytriazole	75.6	9.6	4.7–150	0.989	4.7
Azithromycin	89.8	9.7	1.9–150	0.988	1.9
Erythromycin	90.3	3.1	0.2-150	0.999	<0.2
Prednisolone	107.6	3.2	0.2–150	0.997	0.2
Carbamazepine	90.3	4.0	0.2–150	0.998	<0.2
Clarithromycin	84.6	5.0	0.2–150	0.992	<0.2
Fluoxetine	104.3	8.4	0.2–150	0.998	<0.2
Sertraline	90.8	1.1	0.2–150	0.997	<0.2
Atorvastatin	90.3	4.7	0.2–150	0.998	<0.2
Diclofenac	82.7	1.3	0.5–150	0.999	0.5
Criteria	**75–125%**	**<15%**	**-**	**> 0.990**	**-**

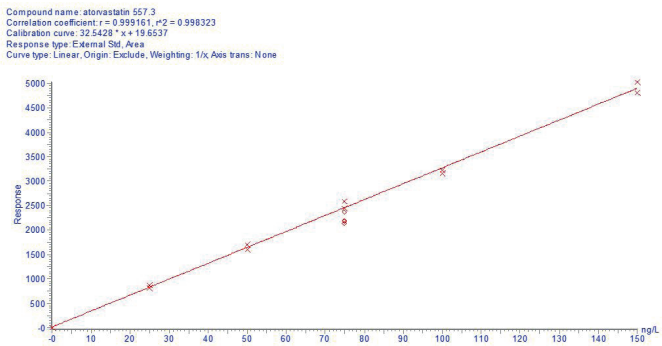

Figure 3. Typical calibration curve for atorvastatin.

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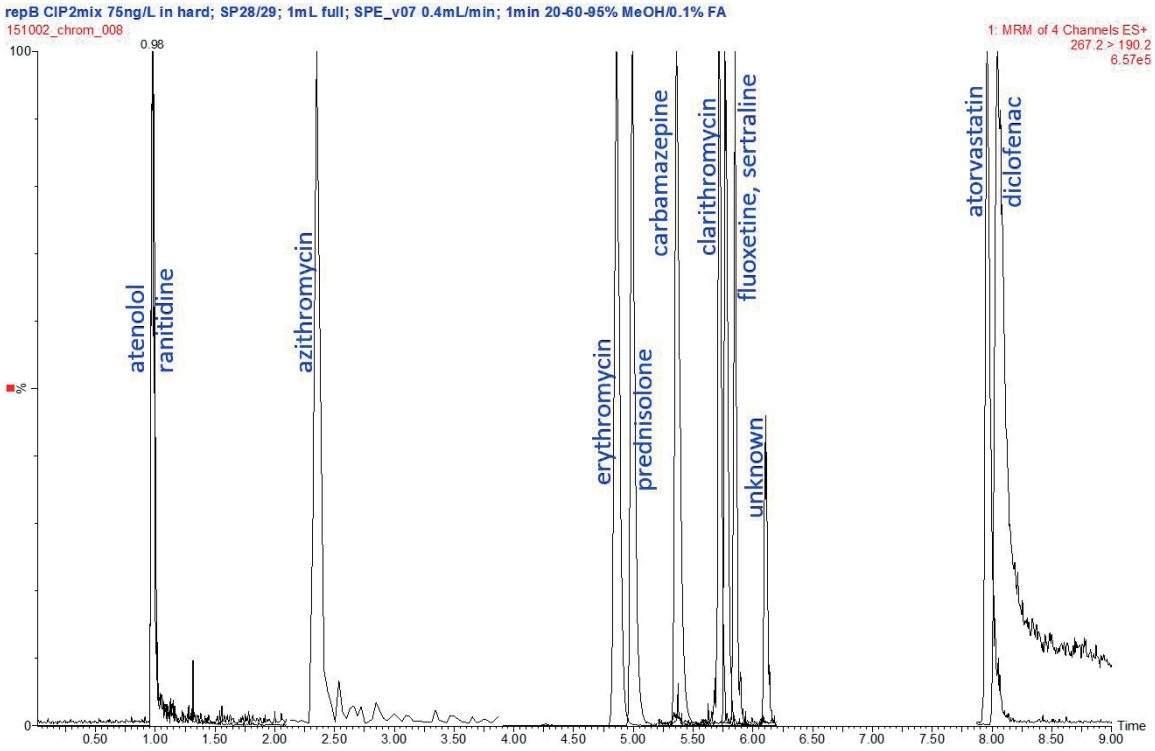

Figure 4. Overlaid SPE-LC-MS/MS MRM Chromatograms of Pure Standards at 75 ng L^{-1}

- The Biotage® EVOLUTE EXPRESS ABN On-line SPE cartridge provided reproducible pre-concentration of pharmaceuticals with acidic, neutral and basic functionality, and a range of polarity.
- The CTC PAL system allowed fully automated, sensitive SPE-LC/MS/MS analysis of the analytes
- Limits of detection ranges from <0.2ng L^{-1} to 4.7 ng L^{-1}
- Repeatability (n=4) ranges from 1.1–9.7% @ 75 ng L^{-1}
- Time per run was 26.5 mins

Ordering Information

Part Number	Description	Quantity
OSPE-620-32150	EVOLUTE® EXPRESS ABN On-line SPE Cartridge 30 x 2.1 mm	1

EUROPE
Main Office: +46 18 565900
Toll Free: +800 18 565710
Fax: +46 18 591922
Order Tel: +46 18 565710
Order Fax: +46 18 565705
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Support Fax: + 46 18 56 57 11
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To locate a distributor,
please visit our website
www.biotage.com

Part Number: AN862

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