

Pharmaceutical Contaminant Screen in Drinking Water and Surface Water by Direct Online Analysis

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Overview

- This online method strives to approximate the off-line EPA method 1694 for PCPP in water.
- Analytes are concentrated on a Thermo Scientific TurboFlow Cyclone-P polymer column, eluted by gradient through a Thermo Scientific Hypersil GOLD C18 HPLC column for final separation, and detected by ESI-MS-MS
- Recoveries achieved by this method typically range from 75 to 95% for most analytes.
- Method run time is 19 minutes.

Introduction

TurboFlow™ Chromatography (TFC) has been used for direct injection of biological fluids onto LC/MS systems without pre-extraction for a decade. This same methodology can also be used for online pre-concentration of water samples. We have developed an on-line method screen for common pharmaceutical compounds found in drinking and surface water.

Methods

Sample Preparation

Type 1 reagent grade water was used as matrix for standards and QCs. Stock solution was prepared for all analytes in methanol:water at 0.100 mg/mL. Stocks were spiked into reagent grade water and that was used as a spiking solution for calibration standards and quality controls (QCs). A 15-point standard curve at 0.0610, 0.122, 0.244, 0.488, 0.977, 1.95, 3.91, 7.81, 15.6, 31.3, 62.5, 125, 250, 500, and 1000 pg/mL and 4 QC points at 7.81, 31.3, 125, and 500 pg/mL were analyzed.

Sample Analysis

Sample concentration was accomplished by on-line passage through a polar polymer-based TurboFlow column. This was followed by organic elution to an end-capped silica-based mixed mode RP analytical column and gradient elution to a Heated Electrospray Ionization (H-ESI) MS/MS (Thermo Scientific TSQ Quantum Ultra triple stage quadrupole mass spectrometer) operated in Positive SRM Mode. The total LC-MS/MS method run time was less than 19 minutes. Figure 1 shows the TurboFlow method environment, Figure 2 shows the LC method parameters used, and Figure 3 shows the analyte list.

Simple and straightforward analyte transfer between the extraction (TurboFlow) column and the analytical column allows for a very wide range of analytes.

FIGURE 1. Thermo Scientific Aria TLX System Quick Elute Mode

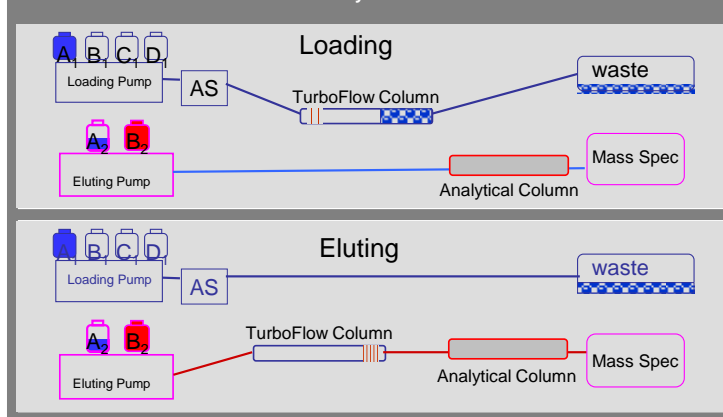


FIGURE 2. Method Parameters

TurboFlow Column: Cyclone-PTM™ (0.5x50mm)
Analytical Column: Thermo Scientific BETASIL Phenyl-Hexyl, 100x3mm, 3um
Aria™ TLX-1 System Plumbing: Quick Elute Mode
Sample Loop Volume: 2.00 mL
Injection Volume: 1.00 mL
Syringe Volume: 5.00 mL
Column and Sample Temperatures: Ambient
Total Method Duration: 18:20 minutes
Aria Operating System Software Version: 1.6.2

Loading Pump Mobile Phases

Mobile Phase A: 1.0 % Formic Acid in Water
Mobile Phase B: 0.3 % Formic Acid in 1:1:1 Isopropanol:Acetonitrile:Acetone
Mobile Phase C: 10mM Ammonium Acetate in Water, pH 9
Mobile Phase D: 50mM Ammonium Acetate in Methanol with 0.1 % Formic Acid

Eluting Pump Mobile Phase

Mobile Phase A: 1.0 % Formic Acid in Water
Mobile Phase B: 0.5 % Formic Acid, 0.04 % TFA in 1:1 Methanol:Acetonitrile

Results

- System directly captured and concentrated analytes.
- Elution gradient through both extraction and separation column reduced analyte loss and thus increased recoveries.
- Injection volume was optimized for 1 mL.
 - Larger volumes of up to 2-5 mL were achieved, but reduced the analyte range.
 - Some analytes were not fully retained on the extraction column at greater volumes.
 - Further optimization is required to take advantage of 2-5 mL injection volumes.
- Result summary (Figure 3)
 - Most standard response curves in water ranged from 7.8 to 1000 pg/mL.
 - Typical regression coefficient from 0.980 to 0.995.
 - Extraction recoveries typically ranged from 75 to 95% for 1 mL injection volume.
- This method provides three critical features.
 - Expanded capture range suitable for a wide range of analytes.
 - Extensive separation between analytes.
 - Dramatic reduction of sample preparation (no SPE, liquid-liquid).

FIGURE 3. Result Summary

ANALYTE	R ²	LLOQ, pg/mL	HLOQ, pg/mL	QC %
Sulfathiazole	0.980	7.8	1000	88
Caffeine	0.990	7.8	1000	74
Codeine	0.990	7.8	1000	77
Acetaminophen	0.985	31.3	1000	73
Ranitidine	0.980	7.8	1000	66
Atenolol	0.994	3.9	1000	89
Albuterol	0.996	0.5	1000	85
Cotinine	0.994	15.6	1000	74
Metformin	0.990	15.6	1000	81
Progesterone*	0.970*	62.5	1000	82
Warfarin	0.990	3.9	1000	78
Penicillin	0.990	31.3	1000	83
Imipramine	0.980	15.6	1000	84
Carbamazepine	0.997	15.6	1000	91
Verapamil	0.980	15.6	1000	66
Diltiazem	0.980	7.8	1000	87
Doxepin	0.980	15.6	1000	73
Trazodone	0.990	7.8	1000	85
Lidocaine	0.993	7.8	1000	90
Pindolol	0.990	3.9	1000	91

* Progesterone ionizes poorly with ESI and is better suited to APCI.

Figure 4. Example chromatogram of five analytes and internal standards at 500 pg/mL in water standard sample.

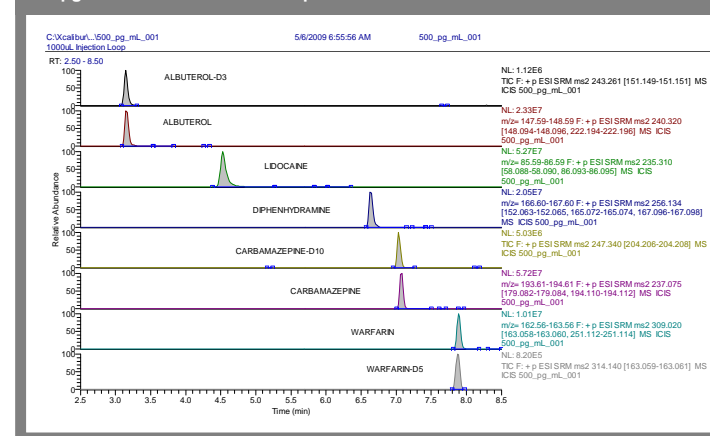


Figure 5. Example chromatogram of six analytes at 15.6 pg/mL in water.

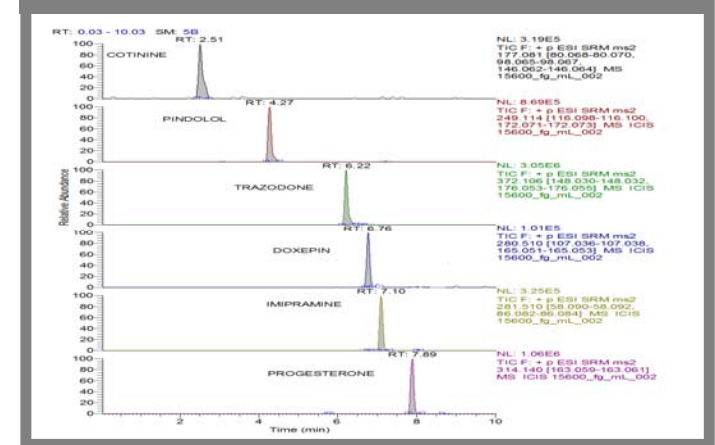


Figure 4 and 5 show typical chromatograms. Even though the gradient runs through both the extraction and separation columns, chromatography remains excellent. More separation can be achieved with a longer column.

Most analytes were not found in local drinking water, with the exception of metformin possibly present in the 10 – 40 pg/mL range. Other compounds show trace levels below, 5 pg/mL which could not be quantified. Lower detection limits are needed to achieve detection below 1 pg/mL.

Future research:

- This method did not adequately capture antibiotics. Recoveries were low and chromatography was not adequate. It may be possible to add major antibiotics to this method by including a second extraction column, such as the Thermo Scientific TurboFlow Cyclone MAX.
- It should be possible to reach fg/mL detection limits with a high-performance MS such as the Thermo Scientific TSQ Vantage triple stage quadrupole mass spectrometer.

Conclusions

- An on-line concentration method was developed for PCPP in water matrix.
- The method is suitable for a wide range of compounds (log D -2 to +4)
- System configuration of Quick Elute Mode with an analytical column allows for an expanded range of analytes.
- The method is quite sensitive, with an LLOQ of 7.8 pg/mL for most analytes, which compare favorably with the EPA1694 method requirements.

Reference

- EPA Method 1694: Pharmaceuticals and Personal Care Products in Water, Soil, Sediment, and Biosolids by HPLC/MS/MS, December 2007.

Acknowledgements

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