Online SPE Multi-Cartridge Kit for Fast and Easy Method Development

The Agilent 1200 Infinity Series Online SPE Solution

Technical Overview

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Abstract

With the Online SPE Multi-Cartridge Kit, Agilent now offers an upgrade kit for easy and fast method development for the Agilent 1200 Infinity Series Online SPE Solution featuring the Agilent 1290 Infinity Flexible Cube. Up to six cartridges with different stationary phases can be installed on the system, which makes it easy to identify the best trapping column for the compounds of interest.

The Multi-Cartridge Kit increases the productivity of the system, but still offers the flexibility of direct injections through valve switching.

Agilent Technologies

Introduction

For the analysis of compounds dissolved in water, a preconcentration and cleaning step is often necessary. The primary method of choice is solid phase extraction (SPE). In addition to the enrichment of analytes, the advantages of an SPE procedure are: reduced interference and matrix effects in the detector (for example, mass spectrometer), enhanced analytical column lifetime, and robustness of the system. Unfortunately, the offline SPE procedure is known to be time-consuming and error-prone.[1]

A more convenient alternative to offline SPE is online SPE. Online SPE uses an approach similar to offline but reduces time and labor, because of the online enrichment. For this purpose, Agilent has developed different online SPE kits with the Agilent 1200 Infinity Series Online SPE Solution, based on the Agilent 1290 Infinity Flexible Cube.

The available kits: Online SPE Kit (G4742A)[2], Online SPE Direct Injection Kit (G4744A)[3] and High Volume Injection Kit (G4745A)[4], offer many possibilities with automation and flexibility. The new Online Multi-Cartridge Kit (G4743A) makes method development for online SPE cartridges easier.

The Online Multi-Cartridge Kit permits selection of up to six SPE cartridges of different materials. Hence, this setup makes it quick and easy to find the most suitable trapping column for the analytes. In combination with the Direct Injection Kit, the Online Multi-Cartridge Kit still offers the flexibility to run direct injection methods with the same system, and without replumbing.

This Technical Overview shows the development of an online SPE method for five estrogens in drinking water with six different trapping columns. The enrichment and analysis of each trapping column was compared to a direct injection run. The trapping column that compared best to the direct injection run was then chosen for the online enrichment process.

Experimental and Instrumentation

All experiments were carried out on an Agilent 1200 Infinity Series Online SPE System comprising:

- Agilent 1290 Infinity Flexible Cube G4227A with two 2-position/10-port valves:
  - Online SPE Starter Kit: G4742A (includes one 2-position/10-port valve, 600 bar)
  - Online SPE Direct Injection Kit: G4744A (includes one 2-position/10-port valve, 600 bar)
  - Online SPE Multi-Cartridge Kit: G4743A (includes one 6-position/14-port valve)
- Agilent 1290 Infinity External Valve Drive G1170A
- Agilent 1260 Infinity Binary Pump G1312B and LAN card G1369C
- Agilent 1260 Infinity Standard Autosampler G1329B with 900 µL head (G1313-60007) and 1290 Infinity Thermostat G1330B
- Agilent 1290 Infinity Thermostatted Column Compartment G1316C
- Agilent 6460A Triple Quadrupole LC/MS System with Agilent Jet Stream technology

Software

- Agilent MassHunter data acquisition for Triple Quadruple Mass Spectrometer, Version 06.00
- Agilent MassHunter Optimizer software, Version 06.00
- Agilent MassHunter Source and iFunnel Optimizer software, Version 06.00
- Agilent MassHunter qualitative software, Version 06.00
- Agilent MassHunter quantitative software, Version 06.00

Consumables

Table 1 lists six trapping columns with different SPE materials. In general, guard columns can also be used as trapping columns, such as numbers 2–6 in Table 1.

Method

1290 Infinity Flexible Cube configuration:

Left 2-position/10-port valve: position 1 → 10 (online SPE), 1 → 2 (direct injection)

Right 2-position/10-port valve: position: 1 → 10 (connection to 6 position/14 port valve), 1 → 2 (capillary for cleaning the system)

Infinity 1290 External valve drive with 6-position/14-port valve: position 1–6 for cartridges 1–6 (Table 1).

Table 1. Trapping columns tested for optimal enrichment of analytes.

No.	Trapping columns	Order no.
1	Agilent BE ONLINE PLRP-S 2.1 × 12.5 mm, 15-20 µm	(p/n 5982-1271)
2	Agilent ZORBAX Eclipse plus C18, 2.1 × 12.5 mm, 5 µm	(p/n 821125-936)
3	Agilent ZORBAX Eclipse plus C8, 2.1 × 12.5 mm, 5 µm	(p/n 821125-937)
4	Agilent ZORBAX Eclipse plus Phenyl-Hexyl 2.1 × 12.5 mm, 5 µm	(p/n 821125-938)
5	Agilent ZORBAX 300SB-C3, 2.1 × x 12.5 mm, 5 µm	(p/n 821125-924)
6	Agilent ZORBAX Bonus RP 2.1 × 12.5 mm, 5 µm	(p/n 821125-928)

Table 2. HPLC method for direct injection and online SPE mode.

	Direct injection	Online SPE
Mobile phase	A) water + 0.2 mM ammonium fluoride B) methanol:acetonitrile (65:35, v:v)	A) water + 0.2 mM ammonium fluoride B) methanol:acetonitrile (65:35, v:v)
Gradient	0–1 minute 10 % B, 1–10 minutes 10–100 % B 10–14 minutes 100% B Post time 12 minutes	0–4 minutes 10 % B, 4 –14 minutes 10–100 % B Post time 12 minutes
Flow rate	0.4 mL/min	0.4 mL/min
Injection volume	225 µL	900 µL
Sample temperature	5 °C	5 °C
LC column	Agilent Poroshell 120 Phenyl Hexyl 3.0 × 50 mm, 2.7 µm (p/n 699975-312)	Agilent Poroshell 120 Phenyl Hexyl 3.0 × 50 mm, 2.7 µm (p/n 699975-312)
Column temperature	30 °C	30 °C
Enrichment cartridges	not applicable	see Table 1

Table 3. Timetable setup for an online SPE method with the Agilent 1290 Infinity Flexible Cube.

Time (min)	Function	Parameter
0	Pump for volume	Pump 3 mL, flow: 1 mL/min Channel A1 (water)
3.2	Right valve change position	Change position 1 → 2
3.3	Pump for time	Pump 2 mL, flow: 1.5 mL/min Channel A2 (acetonitrile)
5	Pump for time	Pump 3 mL, flow: 1.5 mL/min Channel A1
8	Right valve change position	Change position 1 → 10

System configuration and principle of operation

The Agilent 1200 Infinity Series Online SPE Solution offers a method development kit for online SPE cartridges. This Online SPE Multi-Cartridge Kit (G4743A) is based on the Online SPE Kit (G4742A) and is recommended in combination with the Direct Injection Kit (G4744A). It adds a column selection valve (6-position/14-port valve head, 600 bar) for SPE method development. This 6-position/14-port valve is installed next to the 1290 Infinity Flexible Cube in an external valve drive. The Flexible Cube itself hosts two 2-position/10-port valves to support direct injections and online SPE (Figure 1).

On the column selection valve, up to six online SPE cartridges with different SPE materials can be installed for method development. Agilent offers one specialized online SPE material (PLRP-S) and different pre-columns with a wide range of different materials (Table 1), which can be also used as SPE cartridges for sample enrichment.

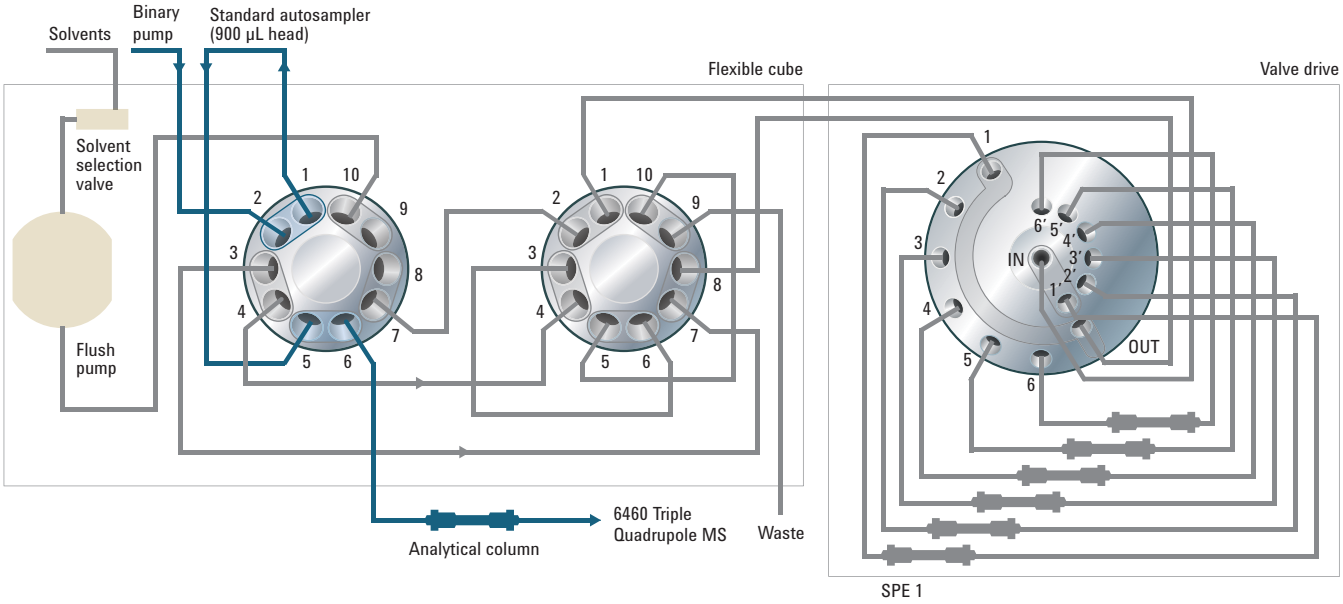

Figure 1. The Agilent 1290 Infinity Flexible Cube offers the possibility of direct injections and online SPE. Here, the flow path of the direct injection run is shown. In this position, the right 2-positon/10-port valve and the additional 6-column selection valve in the external valve drive are disabled and not busy.

With the built-in solvent selection valve in the 1290 Infinity Flexible Cube, up to three solvents can be used to condition and to regenerate the cartridges (Figure 2). Also, the right 2-position/10-port valve can be switched between online SPE and bypass, for cleaning the system.

Finally, the system is coupled to an Agilent 6460 Triple Quadrupole LC/MS System.

This setup enables rapid and convenient online SPE method development with up to six different materials and saves time and labor.

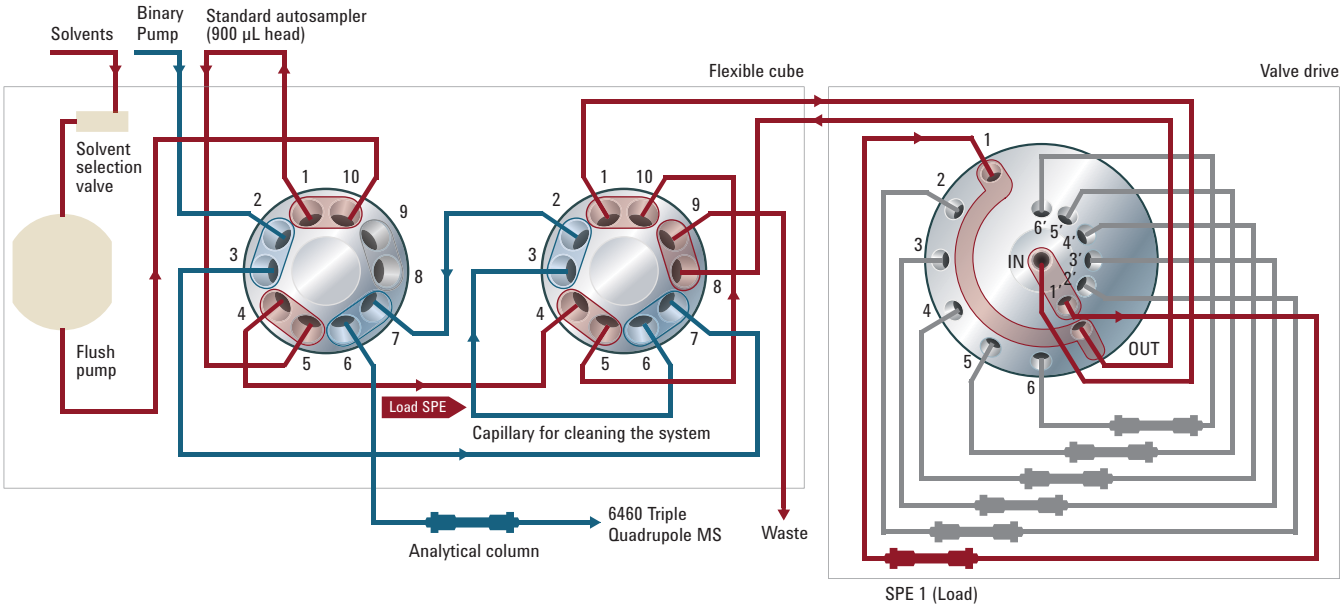

Figure 2. For online SPE, the flow path of the Agilent 1290 Infinity Flexible Cube goes through the left and right 2-position/10-port valve and afterwards to the 6-column selection valve. One out of the six cartridges is loaded with the sample.

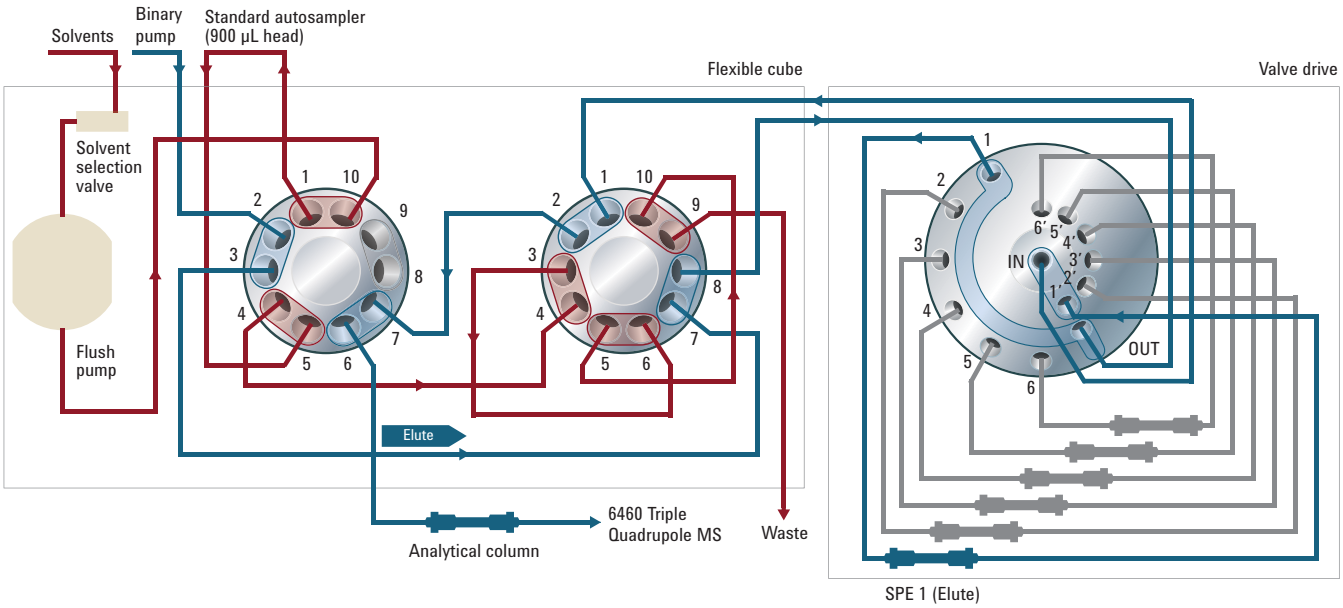

Figure 3. To elute the loaded cartridge in the previous step (Figure 2), the right 2-position/10-port valve is switched.

Method

The Multiple Reaction Monitoring (MRM) triple quadrupole MS method was developed using Agilent MassHunter Optimizer software. Direct injections were used for all estrogen standards (100 ng/µL). For every compound, fragmentor voltage, cell accelerator voltage, and collision energy were optimized for two MRM transitions. The suite of five estrogens is listed in Agilent Technologies Application Note: 5991-3440EN[5].

Samples

Tap water was spiked at two levels (50 and 200 ng/L) with an equimolar mixture of five estrogens. Six consecutive runs were performed for each trapping column and direct injection.

Chemicals

All solvents used were LC/MS grade. Acetonitrile and methanol were purchased from Merck, Germany. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with LC-Pak Polisher and a 0.22-µm membrane point-of-use cartridge (Millipak, EMD Millipore, Billerica, MA, USA). Ammonium fluoride was purchased from Sigma Aldrich, St. Louis, MO, USA.

All estrogen standards were purchased from Dr. Ehrenstorfer GmbH, Germany at a concentration of 100 ng/µL in acetonitrile.

Results and Discussion

To find the best suited trapping column for five estrogens in drinking water, six different SPE cartridges (Table 1) and one direct injection were compared for chromatography, recovery, and area relative standard deviation (RSD).

The same chromatographic conditions were applied for the direct injection and online SPE analysis. The only parameter that was changed between the online SPE runs was the trapping column (Table 1).

Chromatography

The chromatographic effects of different trapping columns are shown in the total ion chromatogram (TIC) in Figure 4 .

Base peak separation was shown for all trapping columns except PLRP-S. PLRP-S showed low sensitivity and peak tailing for all compounds. An example of peak tailing is shown in Figure 5 for 17-α-ethinylestradiol trapped on an Agilent ZORBAX Eclipse plus C18 cartridge and Agilent BE ONLINE PLRP-S.

Good peak shape was shown for all other trapping columns. In addition, Bonus RP showed low sensitivity for the measured estrogens as shown in Figure 4.

Due to the chromatographic results, the PLRP-S and the Bonus RP cartridges were disregarded for further investigations.

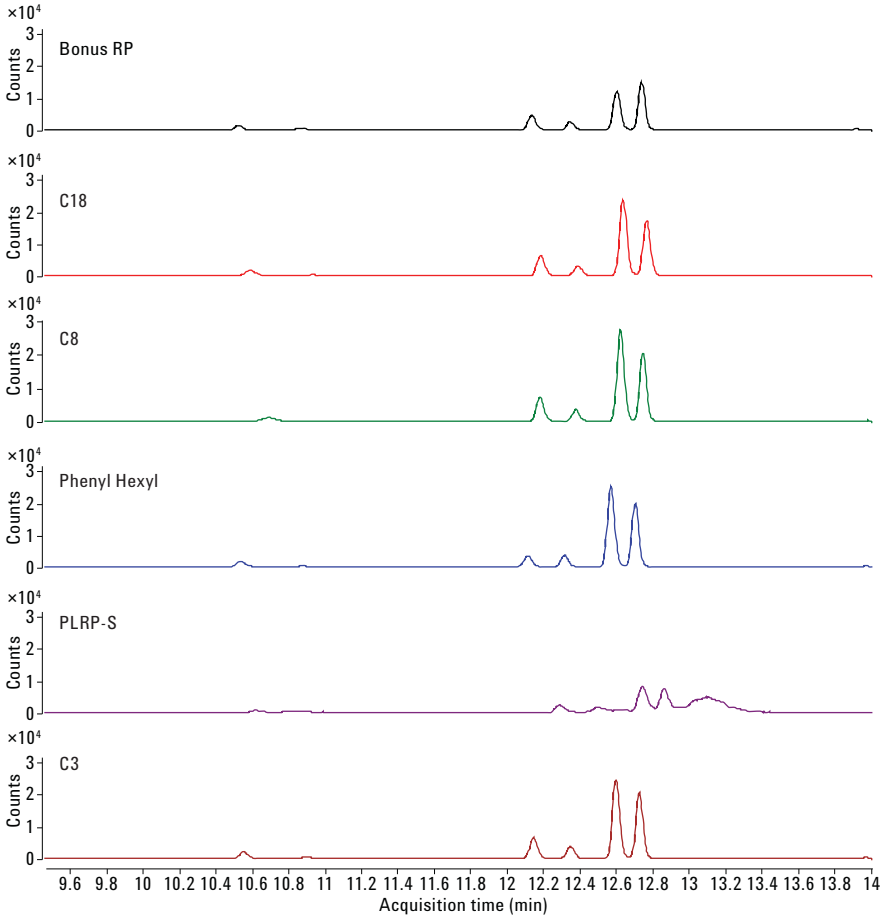

Figure 4. TIC chromatogram for six trapping columns and same chromatographic conditions.

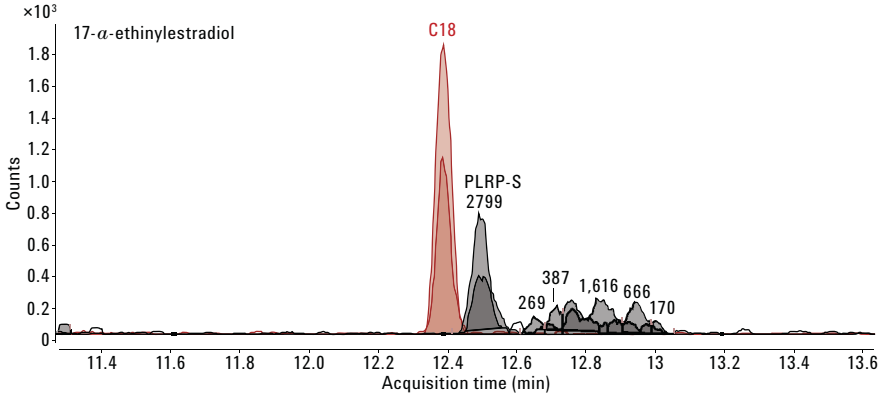

Figure 5. MRM chromatogram of 17-α-ethinylestradiol trapped on an Agilent ZORBAX Eclipse plus C18 and Agilent BE ONLINE PLRP-S cartridge. While the analyte shows considerably tailing with BE ONLINE PLRP-S, slim peaks are observed for C18.

Recovery

Recovery data of the SPE cartridges were determined by comparing the peak areas of a direct injection (225 µL of 200 ng/L) to an online SPE injection (900 µL of 50 ng/L) on one of the cartridges with the same concentration on column.

For the evaluation of the best-suited SPE cartridge, the recovery data, area RSD, and signal-to-noise ratio (S/N) were determined on the example of three compounds (estriol, 17-α-ethinylestradiol, and estrone) in Table 4.

The recovery, area RSD, and S/N data of the direct injection and trapping columns demonstrated that the ZORBAX Eclipse C18 cartridge was most comparable to the direct inject data and is, therefore, perfectly suited for the analysis of estrogens in drinking water. The deviation of the recovery for all compounds showed less than 25 %, and area RSD was < 6 % for six consecutive runs for all measured compounds.

Table 4. Recovery, area RSD and S/N data are shown for estriol, 17-α-ethinylestradiol, and estrone (n = 6).

	Estriol			17-α- Ethinylestradiol			Estrone		
Column	Recovery (%)	Area RSD (%)	S/N	Recovery (%)	Area RSD (%)	S/N	Recovery (%)	Area RSD (%)	S/N
Direct injection	100	6.2	47	100	5.4	110	100	1.7	1,040
Agilent ZORBAX Eclipse plus C18	125	5.92	27	102	5.05	89	88	3.4	480
Agilent ZORBAX Eclipse plus C8	119	10.28	60	101	12.55	136	98	1.27	764
Agilent ZORBAX Eclipse plus Phenyl Hexyl	142	6.1	70	112	4.96	59	91	2.45	401
Agilent ZORBAX 300 SB C3	134	2.1	65	114	5.28	181	93	3.41	843

Figure 6 shows a chromatogram of five estrogens, trapped on an Eclipse C18 cartridge.

Conclusion

The Online SPE Multi-Cartridge Kit permits the use of six different trapping columns in the system for a productive, convenient, and rapid SPE method development. With the Online SPE Multi-Cartridge Kit, it is easy to find the best-suited trapping column for demanding compounds.

The Online SPE Multi-Cartridge Kit allows, in combination with the Direct Injection Kit, not only method development but also direct injection without replumbing the system.

In this Technical Overview, six cartridges of different materials were tested under the same chromatographic conditions. The results from the cartridges were compared to the direct injection with respect to recovery, area RSD, and S/N. The Agilent ZORBAX Eclipse plus C18 trapping column gave the best performance for estrogens in drinking water. It was shown that the variation of chemistry of trapping columns leads to different sensitivity, resolution, peak shape, and area RSD.

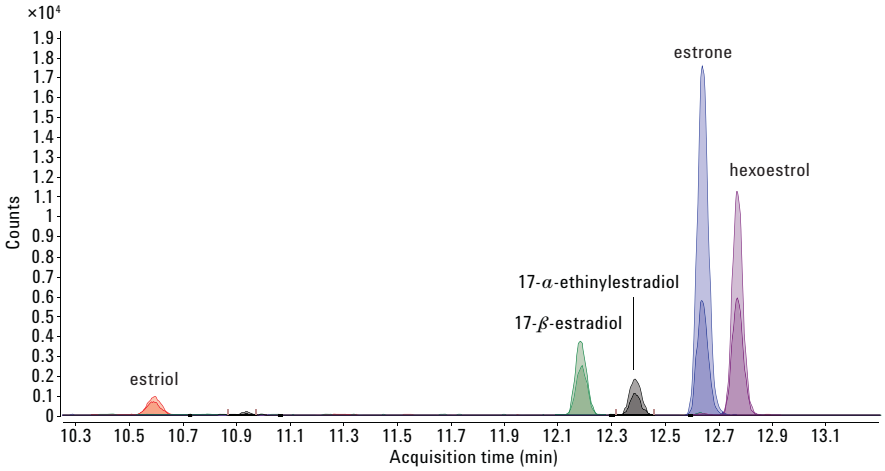

Figure 6. Chromatogram for five estrogens in drinking water (50 ng/L) with online SPE, trapped on an Agilent ZORBAX Eclipse plus C18 SPE cartridge.

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