Application Note

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Analysis of Sulfonamide Drugs in Water Using Agilent Bond Elut HLB Cartridges and LC/MS/MS

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Abstract

This study developed and validated a method for quantitative analysis of 19 sulfonamide (SA) drugs and metabolites in water using Agilent Bond Elut HLB SPE cartridges by LC/MS/MS detection. The method delivered a reliable solution with excellent recoveries and producibility for SA analysis in water, and can be extended to other drug contaminant detection in water.

Introduction

Hydrophilic-lipophilic balanced (HLB) reversed-phase solid phase extraction (SPE) is one of the most widely used SPE products in many applications for environmental, biological, and food analysis. Bond Elut HLB sorbent is composed of monodisperse divinylbenzene and N-vinylpyrrolidone copolymer based on a specific ratio. The hydrophobic divinylbenzene head retains hydrophobic targets well, and the hydrophilic N-vinylpyrrolidone head retains polar compounds efficiently. This SPE sorbent provides good retention for a broad range of compounds from polar, to intermediate polar, to nonpolar compounds, and from acidic, to neutral, to basic compounds. This application note demonstrates the use of Bond Elut HLB 6 mL, 500 mg cartridges for the analysis of 19 sulfonamide drugs in water.

Experimental

Standards and reagents

All the targeted standards were from Alta Scientific Ltd. (Tianjing, China), including 19 sulfonamide drugs and four isotopic internal standards (ISTDs). LC/MS-grade methanol (MeOH) was from Merck. Formic acid, ammonium hydroxide, and ammonium acetate were purchased from Sigma-Aldrich.

Equipment and supplies

- Bond Elut HLB cartridges, 6 mL, 500 mg (part number 5610-2147)
- Agilent Captiva Premium nylon syringe filter, 0.2 µm, 15 mm (part number 5190-5088)
- Agilent Vac Elut SPS 24 manifold with collection rack for 10 × 75 mm test tubes (part number 12234003)

Table 1. HPLC and MS conditions.

HPLC conditions	
Column	Agilent InfinityLab Poroshell 120 EC-C18, 100 × 2.1 mm, 2.7 µm (p/n 695775-902)
Flow Rate	0.3 mL/min
Column Temperature	35 °C
Injection Volume	2 µL
Mobile Phase	A) Water with 0.1% acetic acid B) Methanol
Gradient	Time (min) %A %B 0 90 10 8.0 60 40 12.0 35 65 13.0 5 95 16.0 5 95
Post Time	3 min
MS conditions	
Gas Temperature	325 °C
Gas Flow	6 L/min
Nebulizer	30 psi
Sheath Gas Heater	350 °C
Sheath Gas Flow	11 L/min
Capillary	3,500 V (+)
Nozzle Voltage	500 V (+)
Data Acquisition	MRM as shown in Table 2.

Table 2. Target analytes MRM conditions.

No.	Analyte	Precursor Ion (*m/z*)	Fragmentor (V)	Quant Product Ion (*m/z*)	CE (V)	Qual Product Ion (*m/z*)	CE (V)
1	Sulfacetamide	215.2	65	156	7	108	20
2	Sulfadiazine	251.3	100	156	16	92	32
3	Sulfathiazole	256	100	156.1	14	65.2	56
4	Sulfapyridine	250.3	110	91.9	32	156	16
5	Sulfamerazine	265.3	110	92	32	65.2	58
6	Trimethoprim	291.3	120	230.1	26	261	28
7	Sulfamethoxypyridazine	281.3	100	156	16	92.2	32
8	Sulfamoxole	271.3	90	92.1	28	65.1	56
9	Sulfadimidine	279.3	100	65.2	64	92.1	32
10	Sulfameter	281.3	110	156	16	92.2	34
11	Sulfachloropyridazine	285	100	156	14	92	36
12	Sulfamethoxazole	254.3	100	65.2	54	156	16
13	Sulfamonomethoxine	281.3	70	156	18	92.2	34
14	Sulfisoxazole	268.3	100	155.9	12	92.1	30
15	Sulfadoxine	311.4	130	156	18	92	34
16	Sulfabenzamide	277.2	80	156	12	108	28
17	Sulfaphenazole	315.4	130	65	78	92	43
18	Sulfadimethoxine	311.4	130	156	22	92	38
19	Sulfaquinoxaline	301.4	110	156.1	16	92.1	36
IS 1	$^{13}C_6$-Sulfadiazine	256	110	162	17	–	–
IS 2	$^{13}C_3$-Trimethoprim	294	120	230	38	–	–
IS 3	$^{13}C_6$-Sulfadimethoxine	317	130	162	21	–	–
IS 4	$^{13}C_6$-Sulfamoxole	277	90	162	28	–	–

Instrument method

The samples were run on an Agilent 1290 Infinity II LC system consisting of an Agilent 1290 Infinity II binary pump (G7120A), an Agilent 1290 Infinity II multisampler (G7167B), and an Agilent 1290 Infinity II multicolumn thermostat (G7116B). The UHPLC system was coupled to an Agilent 6470A triple quadrupole LC/MS system equipped with an Agilent Jet Stream Electrospray ionization source. Agilent MassHunter Workstation software was used for data acquisition and analysis.

Sample extraction

Bottled water was purchased from a local store and used as a water blank. Ground surface water was collected from a local river.

The 500 mL of water sample was prepared following the procedure shown in Figure 1.

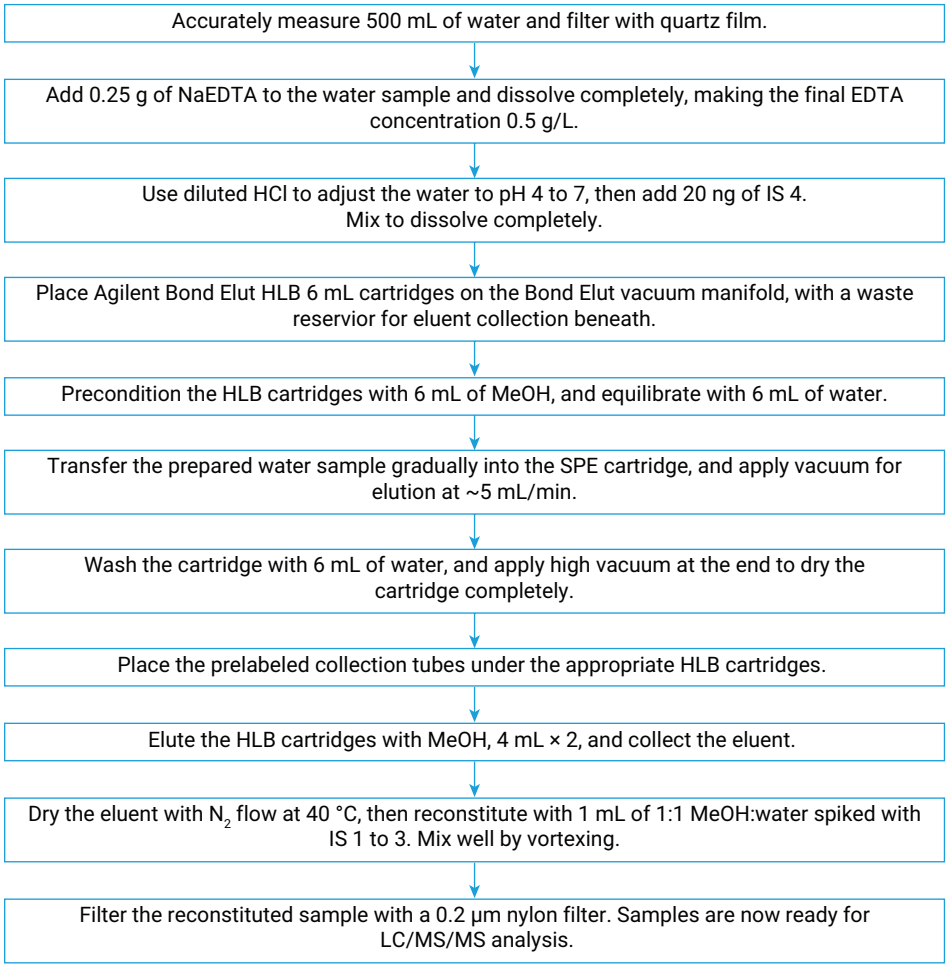

Figure 1. Sample preparation workflow chart.

Results and discussion

Sample preparation optimization

Bond Elut HLB SPE cartridges (500 mg, 6 mL) were used to concentrate the SA targets and purify the water samples in this study.

For the targeted SA compounds, the results showed that the best retention of SA drugs on HLB sorbent was within a pH range of 4 to 7. However, the addition of NaEDTA to the water sample turned the water slightly basic, with a pH ~9, leading to unacceptable results and compromising sulfacetamide and sulfadiazine retention on the HLB sorbent. To improve the retention of these compounds, the water sample was adjusted to pH 4 to 7 using diluted HCl. After targets were retained on the HLB sorbent following sample loading, the elution of the targets was achieved with 3 mL of MeOH, with good recoveries for most targets, but more MeOH (6 mL) was needed to elute sulfamoxole and sulfabenzamide efficiently. Considering the complexity of practical samples, the use of 8 mL of MeOH for elution was used to achieve the best recovery and consistent reproducibility for all of the targets. Figure 2 shows the typical structure of SA targets (A), and the chromatogram of a sample prepared by the developed method (B).

Method sensitivity and calibration linearity

Table 3 shows the method quantitation results. Method calibration was evaluated for the dynamic range of 2 to 200 ng/mL in water. All compounds show excellent linearity, with $R^2 > 0.995$. The limit of detection (LOD) was calculated based on the signal-to-noise ratio (S/N) of the lowest point in the calibration curve for the concentration, which was S/N = 3. The limit of quantitation (LOQ) was decided in the same way, but instead using S/N = 4. The LODs for all 19 SA drugs were within 0.3 to 1.9 ng/L, and limits of quantitation (LOQ) ranged between 1.2 to 7.6 ng/L. The method demonstrated excellent sensitivity.

Method recovery and precision

Method quantitation accuracy and precision were evaluated based on the spiking of 500 mL of blank control water samples at levels of 4, 10, 100, and 200 ng/L. Figure 3 shows target recoveries at four different spiking levels with three replicates at each level. Results demonstrate that the recovery of 74.3 to 118% was achieved for most targets at four spiking levels, except the slight low recoveries (< 65%) of sulfisoxazole at the levels of 4 and 10 ng/L. Precision (RSD%) for all of the targets were between 0.1 and 13.2%.

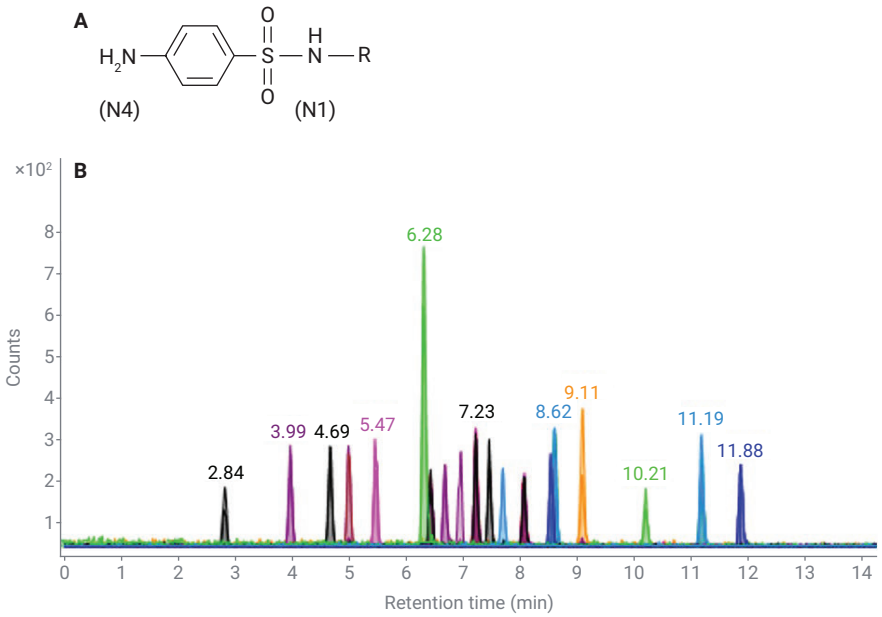

Figure 2. The generic structure of sulfonamides (A) and an overlapped MRM chromatogram of 19 SA targets (B).

Table 3. The 19 targeted SA drugs quantitation results.

Target	Retention Time (min)	Calibration Curve Linearity (R^2)	LOD (ng/L)	LOQ (ng/L)
Sulfacetamide	2.84	0.999	0.29	1.16
Sulfadiazine	3.99	0.999	0.77	3.08
Sulfathiazole	4.69	0.999	1.09	4.36
Sulfapyridine	5.01	0.999	0.83	3.32
Sulfamerazine	5.47	0.999	1.35	5.4
Trimethoprim	6.28	0.999	1.23	4.92
Sulfamethoxypyridazine	6.44	0.999	1.01	4.04
Sulfamoxole	6.69	0.999	0.28	1.12
Sulfadimidine	6.96	0.999	1.24	4.96
Sulfameter	7.24	0.999	1.35	5.4
Sulfachloropyridazine	7.48	0.999	0.86	3.44
Sulfamethoxazole	7.71	0.999	0.98	3.92
Sulfamonomethoxine	8.08	0.999	0.31	1.24
Sulfisoxazole	8.56	0.996	1.51	6.04
Sulfadoxine	8.62	0.999	1.24	4.96
Sulfabenzamide	9.11	0.999	1.88	7.52
Sulfaphenazole	10.21	0.999	0.83	3.32
Sulfadimethoxine	11.19	0.999	0.93	3.72
Sulfaquinoxaline	11.88	0.999	1.26	5.04

Ground surface water analysis

Table 4 shows the spiking recoveries of the 19 targets in ground surface water. The ground surface water was collected from a local river, then spiked with target standard at a level of 100 ng/L. Samples were then prepared at three replicates and quantitated against the established calibration curves. The recovery of all of the targets ranged from 73.4 to 102%, with RSDs between 0.2 and 8.8%. The results confirmed the method applicability to practical, real water sample analysis for sulfonamide drugs with reliable accuracy and precision.

Conclusion

A reliable method has been described for the extraction of 19 sulfonamide drugs from water and analysis by LC/MS/MS using Agilent Bond Elut HLB cartridges. The method was validated to provide excellent calibration linearity with R^2 > 0.995, high sensitivity with LODs of 0.3 to 1.9 ng/L, and LOQs between 1.2 and 7.6 ng/L in water. The spiking recovery for control water and ground surface water was between 61 and 118%, with RSDs < 14%. This method demonstrated excellent accuracy and precision, meeting the requirements of sulfonamide drug analysis in water.

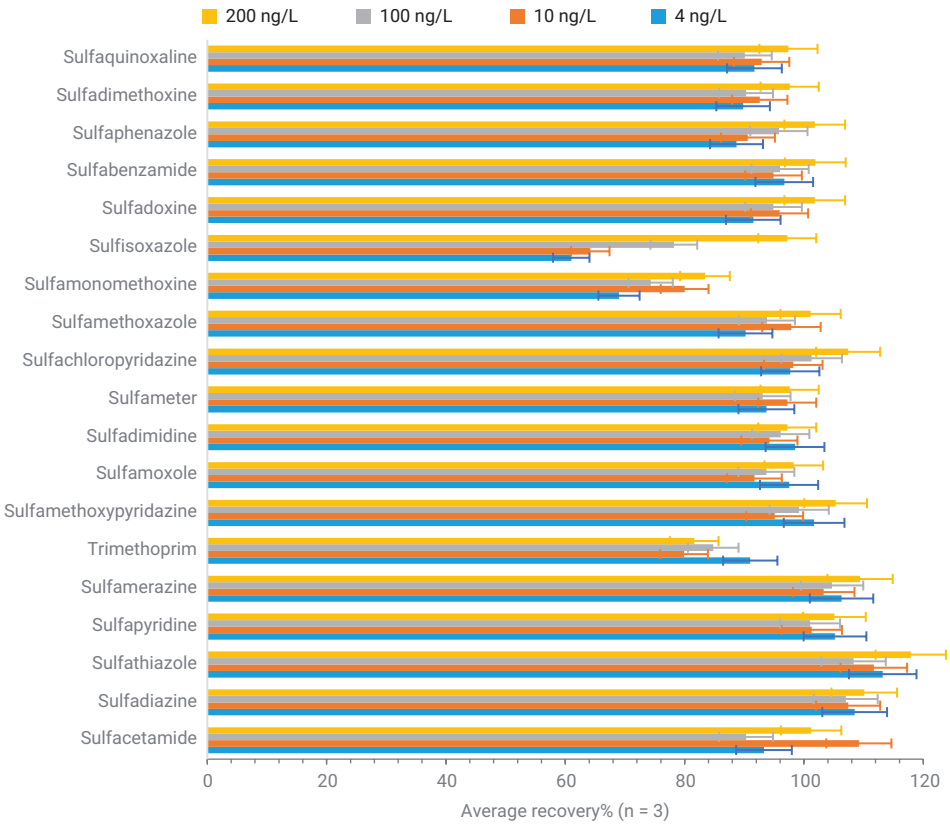

Figure 3. Recoveries of targeted SA drugs in water.

Table 4. Spiking recovery and precision of targets in ground surface water at a level of 100 ng/L (n = 3).

No.	Compound	Average Recovery (%)	RSD (%)
1	Sulfacetamide	95.0	3.6
2	Sulfadiazine	94.4	2.1
3	Sulfathiazole	87.4	0.2
4	Sulfapyridine	85.3	3.4
5	Sulfamerazine	91.8	1.2
6	Trimethoprim	102	0.9
7	Sulfamethoxypyridazine	89.8	1.2
8	Sulfamoxole	88.5	2.0
9	Sulfadimidine	84.0	5.4
10	Sulfameter	94.6	3.11

No.	Compound	Average Recovery (%)	RSD (%)
11	Sulfachloropyridazine	88.6	4.9
12	Sulfamethoxazole	99.5	2.6
13	Sulfamonomethoxine	94.3	5.3
14	Sulfisoxazole	87.5	4.5
15	Sulfadoxine	77.9	6.8
16	Sulfabenzamide	86.8	2.4
17	Sulfaphenazole	78.4	7.0
18	Sulfadimethoxine	78.6	8.2
19	Sulfaquinoxaline	73.4	8.8

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