Application Note 1046

Determination of Phenylurea Compounds in Tap Water and Bottled Green Tea

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Key Words
U.S. EPA Method 532, Pesticides, Water Analysis, On-Line SPE, SolEx HRP

Goal
To develop an efficient HPLC method for the sensitive determination of nine phenylurea compounds in drinking water samples using on-line solid-phase extraction (SPE) and UV detection with method detection limits (MDLs) that meet those reported in U.S. EPA Method 532 and concentration limits set in the European Commission's Council Directive 98/83/EC

Introduction
Phenylurea compounds are widely used as agricultural pesticides. Due to their slow degradation, they are frequently detected in surface waters at concentrations above 0.1 µg/L,[1] which is higher than the European Commission's drinking water limit[2] often used as a quality standard for natural water. U.S. EPA Method 532,[3] the method typically used for the sensitive determination of phenylurea compounds, uses reversed-phase high performance liquid chromatography (RP-HPLC) with UV detection. The structures of the eight phenylurea compounds tested in U.S. EPA Method 532 are shown in Figure 1. U.S. EPA Method 532 requires a sample preparation procedure—off-line SPE—to increase detection sensitivity; however, this procedure is time consuming, requires large volumes of organic solvents, and is deficient in terms of process control.

Equipment
- Thermo Scientific™ Dionex™ UltiMate™ 3000 x2 Dual HPLC system, including:
  - DGP-3600RS Dual Ternary Rapid Separation Pump System with SRD-3600 Integrated Solvent and Degasser Rack
  - WPS-3000TRS Autosampler with a 2500 µL sample loop and a 2500 µL syringe
  - TCC-3000RS or TCC-3000SD Thermostatted Column Compartment equipped with one 2p-6p valve
  - DAD-3000RS Diode Array Detector.
- Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System (CDS) software, version 7.1.
- Thermo Scientific™ Orion™ 2-Star Benchtop pH meter

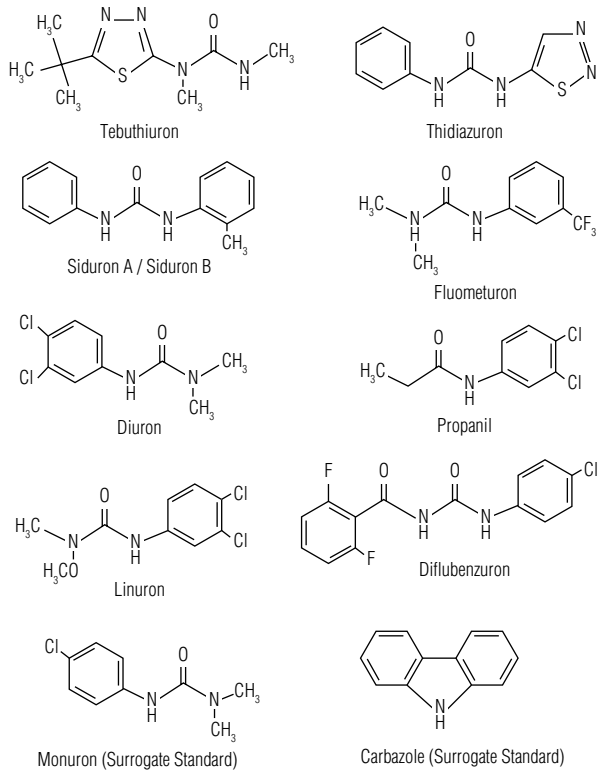

Figure 1. Structures of phenylurea compounds listed in U.S. EPA Method 532 and their surrogate standards.

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Table 1. Preparation of calibration standards.

Volume of SSM[a] (mL)	Volume of SSSM[b] (mL)	Volume of CH_3CN-H_2O Solution (1:49, v/v) (mL)	Final Volume (mL)	Final Concentration	
				Each Phenylurea[c] (µg/L)	Surrogate Standards (µg/L)
0.05	1.0	8.95	10	0.5	50
1.00		8.00		10	
2.00		7.00		20	
5.00		4.00		50	
10.0		0.00		100	

[a] SSM represents "Stock Standard of Phenylurea Calibration Mixture."

[b] SSSM represents "Stock Surrogate Standard of Monuron and Carbazole Mixture."

[c] Phenylureas are tebuthiuron, thidiazuron, fluometuron, diuron, propanil, siduron A, siduron B, linuron, and diflubenzuron; surrogate standards are monuron and carbazole.

Reagents and Standards

- Deionized (DI) water, 18.2 MΩ-cm resistivity
- Acetonitrile (CH_3CN), HPLC grade, Fisher Chemical (P/N AC610010040)
- Methanol (CH_3OH), HPLC grade, Fisher Chemical (P/N AC610090040)
- Ammonium formate ($HCOONH_4$), Fisher Chemical (P/N A666-500)
- Phenylurea Primary Dilution Standard M-532, consists of tebuthiuron (CAS 34014-18-1), thidiazuron (CAS 51707-55-2), fluometuron (CAS 2164-17-2), diuron (CAS 330-54-1), propanil (CAS 709-98-8), siduron (A and B, CAS 1982-49-6), linuron (CAS 330-55-2), diflubenzuron (CAS 35367-38-5), 100 µg/mL each, AccuStandard®
- Phenylurea Surrogate Standard M-532SS, consists of monuron (CAS 150-68-5), and carbazole (CAS 86-74-8), 500 µg/mL each, AccuStandard

Monuron and carbazole were chosen as surrogate standards. Monuron is a phenylurea no longer used in the U.S. In the Figure 3 chromatogram, monuron elutes early and carbazole elutes late.[3]

Stock Standard of Phenylurea Calibration Mixture (SSM)

Dilute 10 µL of Phenylurea Primary Dilution Standard M-532 (100 µg/mL each) to 10 mL with 9.99 mL of methanol. The concentration of each component is 100 µg/L.

Stock Surrogate Standard of Monuron and Carbazole Mixture (SSSM)

Dilute 10 µL of Phenylurea Surrogate Standard M-532SS (500 µg/mL each) to 10 mL with 9.99 mL of methanol. The concentrations of monuron and carbazole are 500 µg/L, respectively.

Working Standard Solutions for Calibration

Prepare five working standard solutions for the calibration with different concentrations by adding the proper amount of stock standard of phenylurea calibration mixture with an acetonitrile/water solution (1:49, v/v). The volumes of each solution needed to make the calibration standards are shown in Table 1.

Sample Preparation

A bottled green tea beverage was purchased from a local market and tap water samples were collected at the Thermo Fisher Scientific™ Shanghai Applications Lab. Filter each sample solution through a 0.45 µm filter prior to direct injection.

Chromatographic Conditions	
For On-Line SPE:	
Cartridge:	Thermo Scientific™ Dionex™ SolEx™ HRP, 12–14 µm, 2.1 × 20 mm, (P/N 074400) Use V-3 Cartridge Holder (P/N 074403)
Mobile Phase:	A: H_2O; B: methanol
Gradient:	0–4 min, 10–100% B; 13 min, 100% B; 13.1–20 min, 10% B
Flow Rate:	1.0 mL/min
Inj. Volume:	2500 µL on the on-line SPE cartridge
For Separation:	
Column:	Thermo Scientific™ Acclaim™ 120 C18, 3 µm Analytical, 3.0 × 150 mm (P/N 063691)
Mobile Phase:	A: 20 mM $HCOONH_4$, B: acetonitrile
Gradient:	0–4 min, 35% B; 4.1 min, 40% B; 7.5–15.8 min, 60% B; 16 min, 35% B
Flow Rate:	0.6 mL/min
Column Temp:	25 °C
Detection:	UV absorbance at 245 nm
Valve Position:	
	0 min, 1_2
	4.0 min, 6_1
	7.8 min, 1_2

Results and Discussion

Evaluations of On-Line SPE

Figure 2 shows a typical flow schematic of on-line SPE, which is directly coupled to the analytical HPLC column using one six-port (2p to 6p) column valve. The filtered sample is injected directly onto the system and delivered to the SPE column for enrichment (1-2 position) using one pump. Simultaneously, the analytical column is equilibrated with another pump. After the analytes are bound to the SPE column and impurities are washed out, the SPE column is switched into the analytical flow path to elute the bound analytes (6-1 position); then the analytes are separated on the analytical column and detected by the UV detector. This method is easily accomplished with the UltiMate 3000 x2 Dual HPLC system.

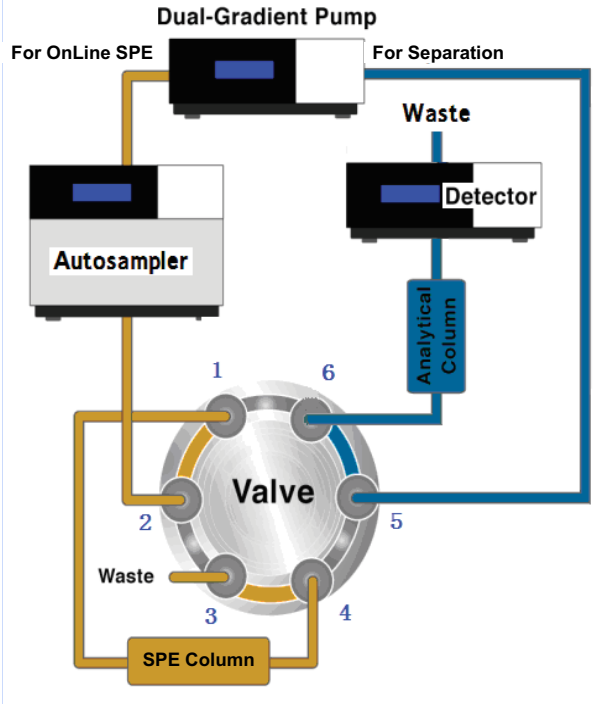

Figure 2. Flow schematic of on-line SPE.

Optimization of Conditions of On-Line SPE

To develop the on-line SPE method, we determined the retention fidelity of the phenylureas on the SPE cartridge, and evaluated the ease of their elution from the cartridge. The elution solvent must be compatible with the subsequent analytical separation. We also determined the volume of sample that could be injected without cartridge overload. The cartridge is more likely to be overloaded by the sample diluent or another component(s) in the sample.

The Dionex SolEx HRP cartridge, which is packed with a divinylbenzene polymer with a hydrophilic bonded layer[4] and which has already been applied to the on-line SPE HPLC analysis of carbamates,[5] and aniline and nitroanilines,[6] was selected as the SPE column here based on its excellent retention properties of phenylureas with different polarities.

Methanol/water and methanol/ammonium formate buffer were evaluated as on-line SPE mobile phases. Experiments showed that the SPE efficiencies were almost the same when using the two mobile phases. Moreover, methanol/water in different proportions (3:7 and 1:9 methanol/water, v/v) was evaluated as well, and there was no obvious difference in term of SPE peak area efficiency. Therefore, the weaker and less expensive mobile phase (methanol/water, 1:9, v/v) was selected.

As in RP-HPLC, sample diluent in on-line SPE HPLC can strongly influence peak shape and sample solubility.[7] Sample diluent can also affect SPE peak area efficiency. Here, a series of acetonitrile/water solvents with proportions of 1:1, 1:4, 1:9 and 1:49 (v/v) were used to prepare standard solutions of phenylureas. The results showed that the best peak shapes for phenylureas were obtained with the proportion of 1:49 (v/v). This proportion allows 50 µL of stock standard solution to be diluted to 2500 µL, which is injected directly onto the on-line SPE cartridge using a 2500 µL syringe matching the sample volume injected.

Reproducibility, Linearity and Detection Limits

Figure 3 illustrates good separation of nine phenylureas and two surrogate compounds following on-line SPE under the specified chromatographic conditions. Here a MS-compatible mobile phase—acetonitrile/ammonium formate buffer—was used instead of an acetonitrile/phosphate buffer, the mobile phase used in U.S. EPA method 532.[3]

For On-Line SPE

Cartridge:	Dionex SolEx HRP, 12–14 µm, 2.1 × 20 mm (Use V-3 Cartridge Holder)
Mobile Phase:	A: H_2O, B: methanol
Gradient:	0–4 min, 10–100% B; 13 min, 100% B; 13.1–20 min, 10% B
Flow Rate:	1.0 mL/min
Inj. Volume:	2500 µL on the on-line SPE cartridge

For Separation

Column:	Acclaim 120 C18, 3 µm Analytical, 3.0 × 150 mm
Mobile Phase:	A: 20 mM $HCOONH_4$, B: acetonitrile
Gradient:	0–4 min, 35% B; 4.1 min, 40% B; 7.5–15.8 min, 60% B; 16 min, 35% B
Flow Rate:	0.6 mL/min
Temperature:	25 °C
Detection:	UV absorbance at 245 nm

Peaks: 5.0 µg/L each

1. Tebuthiuron
2. Thidiazuron
3. Monuron (Surrogate Standard, 20 µg/L)
4. Fluometuron
5. Diuron
6. Propanil
7. Siduron A
8. Siduron B
9. Linuron
10. Carbazole (Surrogate Standard, 20 µg/L)
11. Diflubenzuron

Valve Position: 0 min, 1_2; 4.0 min, 6_1; 7.8 min, 1_2

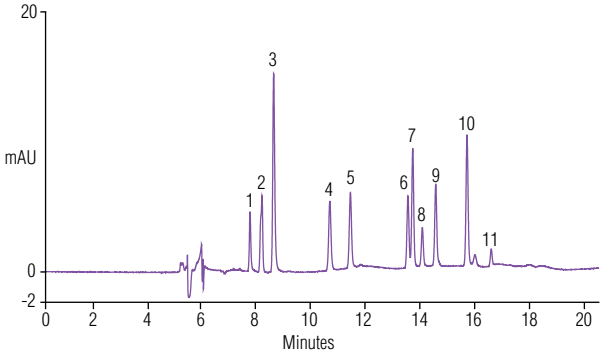

Figure 3. Chromatogram of phenylurea compounds and surrogate standards.

Method precision was estimated using UV detection by making seven consecutive 2500 µL injections of a calibration standard with a concentration of 20 µg/L for each phenylurea compound. The retention time and peak area reproducibilities summarized in Table 2 show good precision.

Calibration linearity for UV detection of phenylureas was investigated by making three consecutive 2500 µL injections of a mixed standard prepared at five different concentrations (i.e., 15 total injections). The external standard method was used to establish the calibration curve and to quantify phenylureas in the drinking and environmental water samples. Excellent linearity was observed from 0.5 to 500 µg/L when plotting the concentration versus the peak area, and the coefficients of determination were all ≥ 0.993 (Table 3).

The method detection limits of each compound for UV detection were calculated by using the equation:

$$\text{Method Detection Limit} = S t_{(n-1,\,1-\alpha=0.99)}$$

The symbol S represents standard deviation of replicate analyses, 'n' represents number of replicates, and $t_{(n-1,\,1-\alpha=0.99)}$ represents Student's value for the 99% confidence level with n – 1 degrees of freedom. Seven replicate injections of reagent water spiked with 2.5 µg/L of the phenylureas standard mixture were used to determine the MDLs. Table 3 summarizes the MDL data, which show excellent method sensitivity with detection limits equivalent to those reported in U.S. EPA Method 532, while meeting the 0.1 µg/L concentration limit set in European Commission's Council Directive 98/83/EC. The 2500 µL injection of this on-line SPE method is equivalent to half the 10 µL injection of the sample prepared from 500 mL of sample following U.S. EPA Method 532.

Table 2. Reproducibility of peak retention time and area.

Analyte	Retention Time RSD	Peak Area RSD
Tebuthiuron	0.05	1.40
Thidiazuron	0.04	0.80
Monuron	0.06	0.28
Fluometuron	0.08	0.78
Diuron	0.07	2.57
Propanil	0.05	0.52
Siduron A	0.05	1.83
Siduron B	0.05	1.07
Linuron	0.04	1.30
Carbazole	0.04	1.64
Diflubenzuron	0.03	1.89

Tap and Bottled Beverage Analysis

Figure 4 compares chromatograms of a green tea sample with the same sample fortified with the mixed phenylureas standard and two surrogate compounds (monuron and carbazole). Four phenylureas—tebuthiuron (Peak 1), monuron (Peak 3), linuron (Peak 9), and diflubenzuron (Peak 11)—were found in the green tea sample. Although monuron (Peak 3) is no long in use in the U.S., it was still detected in the bottled green tea purchased in the Shanghai China market. Three peaks with retention time near that of thidiazuron (Peak 2), propanil (Peak 6), and siduron B (Peak 8), respectively, were found; however, comparison of the UV spectra to those of the standards revealed that the peaks were not phenylureas. Figure 5 shows chromatograms of a tap water sample and the same sample fortified with phenylureas standard and two surrogate compounds. No phenylureas were detected in the tap water sample. The analysis results and related data are summarized in Table 4. These data show excellent spike recovery for each phenylurea, thereby suggesting method accuracy, and demonstrate that this on-line SPE HPLC method provides good selectivity and suitability for the determination of phenylureas in water samples. Larger sample volumes can be injected, but some samples may overload the on-line SPE cartridge, and therefore analyte recovery must be assessed.

Table 3. Calibration data and MDLs.

Analyte	Regression Equation	r^2	Range of Standards µg/L	MDL[a] µg/L	Requirement of U.S. EPA Method 532[b] µg/L	Restriction in 98/83/EC µg/L
Tebuthiuron	A = 0.8764 c + 1.8480	0.9927	0.5–100	0.041	0.071	0.1
Thidiazuron	A = 0.8327 c - 0.4183	0.9979		0.037	0.047	
Fluometuron	A = 1.5523 c - 0.37281	0.9979		0.056	0.027	
Diuron	A = 1.5792 c - 0.6356	0.9976		0.039	0.026	
Propanil	A = 0.3649 c - 0.5374	0.9984		0.043	0.084	
Siduron A	A = 0.3657 c - 0.5347	0.9981		0.088	0.091	
Siduron B	A = 0.7157 c - 0.2570	0.9979		0.088	0.091	
Linuron	A = 0.1618 c - 0.6202	0.9980		0.093	0.067	
Diflubenzuron	A = 0.7559 c - 0.4357	0.9984		0.068	0.033	

[a] The single-sided Student's test method (at the 99% confidence limit) was used for determining MDL, where the standard deviation (SD) of the peak area of seven injections is multiplied by 3.71 to yield the MDL.

[b] Cartridge extraction of a 500 mL water sample, concentrated to about 0.5 mL, then diluted to 1 mL with methanol and 10 µL is injected. Primary Column (C18 stationary phase).

For On-Line SPE
Cartridge: Dionex SolEx HRP, 12–14 µm, 2.1 × 20 mm (Use V-3 Cartridge Holder)
Mobile Phase: A: H_2O, B: methanol
Gradient: 0–4 min, 10–100% B; 13 min, 100% B; 13.1–20 min, 10% B
Flow Rate: 1.0 mL/min
Inj. Volume: 2500 µL on the on-line SPE cartridge

For Separation
Column: Acclaim 120 C18, 3 µm Analytical, 3.0 × 150 mm
Mobile Phase: A: 20 mM $HCOONH_4$, B: acetonitrile
Gradient: 0–4 min, 35% B; 4.1 min, 40% B; 7.5–15.8 min, 60% B; 16 min, 35% B
Flow Rate: 0.6 mL/min
Temperature: 25 °C
Detection: UV absorbance at 245 nm
Samples: a. Green tea
b. Green tea (already 3-fold dilution with water) fortified with phenylurea standards (2.5 µg/L each)
c. Mixture of phenylurea standards (2.5 µg/L each).

Peaks:
1. Tebuthiuron 5.0 µg/L each
2. Thidiazuron
3. Monuron (Surrogate Standard, 20 µg/L)
4. Fluometuron
5. Diuron
6. Propanil
7. Siduron A
8. Siduron B
9. Linuron
10. Carbazole (Surrogate Standard, 20 µg/L)
11. Diflubenzuron

Valve Position: 0 min, 1_2; 4.0 min, 6_1; 7.8 min, 1_2

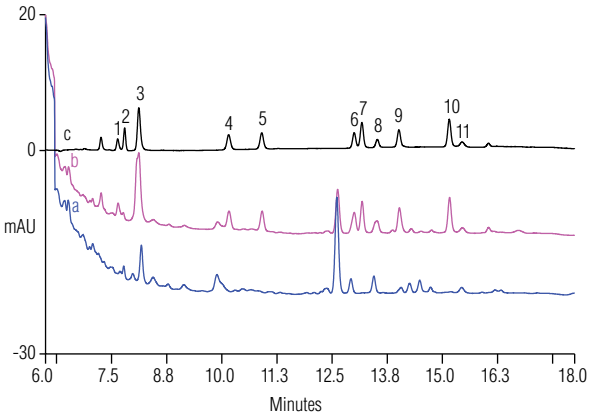

Figure 4. Chromatograms of a green tea sample, the same sample fortified with phenylurea standards, and a mixture of phenylurea standards.

For On-Line SPE
Cartridge: Dionex SolEx HRP, 12–14 µm, 2.1 × 20 mm (Use V-3 Cartridge Holder)
Mobile Phase: A: H_2O, B: methanol
Gradient: 0–4 min, 10–100% B; 13 min, 100% B; 13.1–20 min, 10% B
Flow Rate: 1.0 mL/min
Inj. Volume: 2500 µL on the on-line SPE cartridge

For Separation
Column: Acclaim 120 C18, 3 µm Analytical, 3.0 × 150 mm
Mobile Phase: A: 20 mM $HCOONH_4$, B: acetonitrile
Gradient: 0–4 min, 35% B; 4.1 min, 40% B; 7.5–15.8 min, 60% B; 16 min, 35% B
Flow Rate: 0.6 mL/min
Temperature: 25 °C
Detection: UV absorbance at 245 nm
Samples: a. tap water
b. tap water fortified with phenylurea standards (2.5 µg/L each)

Peaks:
1. Tebuthiuron 5.0 µg/L each
2. Thidiazuron
3. Monuron (Surrogate Standard, 20 µg/L)
4. Fluometuron
5. Diuron
6. Propanil
7. Siduron A
8. Siduron B
9. Linuron
10. Carbazole (Surrogate Standard, 20 µg/L)
11. Diflubenzuron

Valve Position: 0 min, 1_2; 4.0 min, 6_1; 7.8 min, 1_2

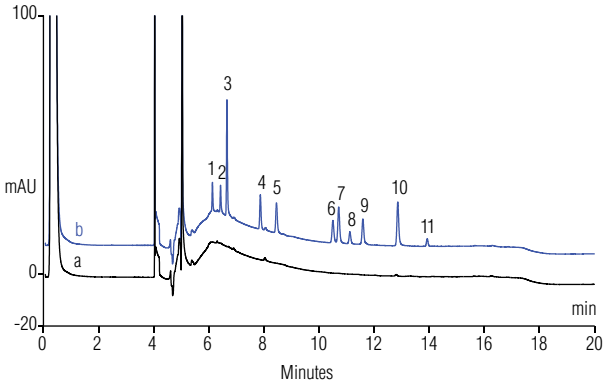

Figure 5. Chromatograms of a tap water sample, and the same sample fortified with phenylurea standards.

Table 4. Analysis results of water samples.

Sample	Bottled Green Tea				Tap Water			
Analyte	Detected µg/L	Added µg/L	Found µg/L	Recovery %	Detected µg/L	Added µg/L	Found µg/L	Recovery %
Tebuthiuron	0.21	2.5	2.75	110	ND	2.5	2.63	105
Thidiazuron	ND[a]		2.10	84			2.21	88
Fluometuron	ND		2.62	105			2.47	99
Diuron	ND		2.62	105			2.65	106
Propanil	ND		2.87	115			2.72	109
Siduron A	ND		2.77	111			2.70	108
Siduron B	ND		2.40	96			2.50	100
Linuron	0.52		2.58	103			2.48	99
Diflubenzuron	2.3		2.55	102			2.55	102
Monuron	7.2[b]	/	/	/		/	/	/

[a] ND represents "not detected."

[b] Estimated value by comparing the peak area of monuron in the green tea sample to that in the mixture of standards (10 µg/L).

Conclusion

This work describes an on-line SPE HPLC with UV absorbance detection method for determining phenylureas in drinking water and commercial bottled beverages. The determination was performed on the UltiMate 3000 HPLC dual-pump system controlled by Chromeleon software. The reduced MDLs using UV detection afforded by the on-line SPE created a convenient method for determining these compounds in drinking water with MDLs meeting the sensitivity of U.S. EPA Method 532 and allowing the detection of concentrations below the 0.1 µg/L limit set in European Commission's Council Directive 98/83/EC without the labor of off-line SPE.

References

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