Fast Separation of Seven Biocides Using an Agilent ZORBAX Rapid Resolution High Definition Eclipse Plus C18 1.8 µm Column

Application note

Environmental

Authors

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Abstract

An Agilent ZORBAX Rapid Resolution High Definition (RRHD) Eclipse Plus C18 1.8 µm column separated seven different biocides rapidly with high resolution. The data presented illustrate the capability of the short 50 mm, 1.8 µm column to maintain high resolution of complex samples at very fast flow rates and high pressures, up to 1020 bar. The high quality separation was maintained when the method run was reduced from 3 minutes to 0.7 minutes by increasing the flow rate from 1.0 mL/min to 1.7 mL/min. The Agilent 1290 Infinity LC System was used because the column pressure just exceeded 1000 bar at this high flow rate. Methylparaben, listed as an echinacea supplement ingredient, was easily identified and a hand sanitizer containing 2-phenoxyethanol and methylparaben were also easily and rapidly identified using the method.

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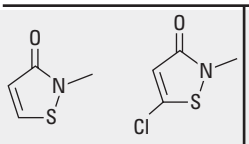
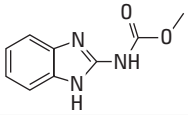
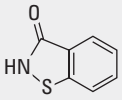
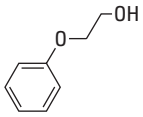
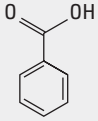
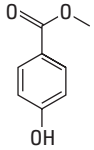
Introduction

Biocides are used to control harmful organisms such as bacteria, fungi and rodents and to help protect health, improve product performance and prevent spoilage. They are found in many areas including medicine, agriculture, forestry, and industry. Due to their potential health risk to humans and the environment, they are highly regulated. In the US, biocides are listed on the national inventory of chemical substances called TSCA. There are also regulations governing the use of biocides on both the federal (EPA) and state level. In Europe, the regulations governing the use of biocides are on a countrywide level. The biocides industry in the European Union (EU) is undergoing a dramatic transformation because the European Commission recently presented draft legislation to achieve a higher level of protection of health and environment to take effect in 2013.

The demand for biocides will continue to grow as public awareness of the benefits of continued hygiene improvements becomes ever more apparent. It is vital therefore to ensure that only biocides safe for use are placed on the market. Because many biocides are synthetic, this work includes the fast separation of seven different synthetic biocides used in different applications. Obtaining results in the shortest time possible without compromising the quality of the results is an area of intense interest for lab analysts, because of the cost and efficiency advantages associated. Table 1 illustrates the biocides investigated in this study, along with their uses.

Table 1. Synthetic Biocides Included in Study and their Uses

Biocide Structure	Name	Uses
	Kathon CG/ICP 1. 2-methyl-4-isothiazonlin-3-one 2. 5-chloro-2-methyl-4-isothiazolin-3-one	Preservative with a wide variety of household and industrial uses.
	3. Carbendazim	Fungicide
	4. 1, 2-Benzisothiazol-3(2H)-one	Used as a preservative in emulsion paints, varnishes, adhesives, washing agents, fuels, and in the paper making process.
	5. 2-Phenoxyethanol	Preservative and bactericide used in vaccines and in dermatological products such as skin creams and sunscreen.
	6. Benzoic Acid	Used as a food preservative and an antimicrobial agent in toothpastes, mouthwashes, cosmetics, and deodorants.
	7. Methylparaben	Antifungal widely used as a preservative for food, drugs, and cosmetics.

Experimental

The biocides were purchased from Sigma Aldrich and include Kathon CG/ICP [containing 0.4% of 2-methyl-4-isothiazolin-3-one (1), 1.2% of 5-chloro-2-methyl-4-isothiazolin-3-one (2)], carbendazim (3), 1,2-benzisothiazol-3(2H)-one (4), 2-phenoxyethanol (5), benzoic acid (6), and methylparaben (7). Kathon was diluted with distilled, deionized water to make a 200-ppm solution of (1) and a 600-ppm solution of (2). Compounds (3-7) were dissolved in acetonitrile (ACN) to make a 200-ppm solution mix. Equal volumes of the Kathon solution and the solution mix were combined to give a 100-ppm stock solution (except for compound (2) that had a concentration of 300 ppm). Six levels of calibration standards were prepared in the range of 2.5 to 20 ppm by dilution of the 100-ppm stock solution in 5% ACN for compounds (1) and (3-7). A three-level calibration was made for compound (2) in the range 7.5 to 23 ppm by dilution of the stock solution with 5% ACN. A standard mix [50 ppm for (1) and (3-7), 150 ppm for (2)] was made by dilution of the stock solution [(100 ppm (1), (3-7) and 300 ppm (2)] with 5% ACN.

Three methods were used to analyze the seven biocides using an ACN/trifluoroacetic acid (TFA) mobile phase adjusting gradient conditions and flow rate. The ACN (CHROMASOLV for HPLC gradient grade, ≥99%) and TFA (≥99%) were purchased from Sigma-Aldrich. The experimental conditions for the three methods are listed in Table 2. Formic acid (98-100%) was from Riedel-de Haën. Formic acid (0.1%) can be substituted for TFA to assist in mass spectral detection.

Table 2. Experimental Conditions for Three, One and 0.7 Minute Methods

All Methods	
Column:	Agilent ZORBAX Rapid Resolution High Definition (RRHD) Eclipse Plus C18 2.1 × 50mm, 1.8-µm, p/n 959757-902
Mobile Phase:	A: Water (0.05 v% Trifluoroacetic acid (TFA) B: Acetonitrile (0.04 v% TFA)
Column Temp:	30 °C
Injection:	1 µL
UHPLC:	Agilent 1290 Infinity LC System
Column Wash:	Column washed with 100% B at end of each run.
Equilibration:	Column equilibrated for two minutes with 5% B before injection.
Three Minute Method – Fast Analysis	
Gradient:	Time (min) 0: 95/5 A/B Time 2.9: 85/15 A/B
Flow Rate:	1.0 mL/min
Detection:	Diode array (DAD) with programmed wavelength switching 275 nm (0 min) 225 nm (1.6 min) 255 nm (2.8 min)
One Minute Method – Faster Analysis	
Gradient:	Time (min) 0: 95/5 A/B Time 0.1: 95/5 A/B Time 0.25: 90/10 A/B Time 1.0: 65/35 A/B Time 1.1: 0/100 A/B
Flow Rate:	1.0 mL/min
Detection:	DAD with programmed wavelength switching 275 nm (0 min) 225 nm (0.8 min) 255 nm (1.08 min)
0.7 Minute Method – Ultra Fast Analysis	
Gradient:	Time (min) 0: 95/5 A/B Time 1.0: 55/45 A/B
Flow Rate:	1.7 mL/min
Detection:	DAD with programmed wavelength switching 275 nm (0 min) 225 nm (0.46 min) 255 nm (0.67 min)

Results and Discussion

An Agilent ZORBAX Rapid Resolution High Definition (RRHD) Eclipse Plus C18 2.1 × 50 mm, 1.8 µm column separated seven different biocides rapidly with high resolution. Three different methods are presented in Figure 1, which show that a high quality separation can be achieved in 3 minutes, and in as short a time as 0.7 minutes. Using the ultrafast, 0.7 minute method, all compounds were baseline-resolved with a minimum resolution factor of 1.8 illustrating the power of sub 2-micron columns to maintain high resolution at fast flow rates. The very fast linear gradient and high flow rate of 1.7 mL/min used with the 0.7 minute method on the 50 mm RRHD column produced pressure slightly over 1000 bar. The RRHD columns are designed for UHPLC systems such as the Agilent 1290 Infinity operating at pressures up to 1200 bar. The Agilent 1290 Infinity LC System was equipped with a diode array detector and the method included programmed wavelength switching to maximize detection response and improve sensitivity.

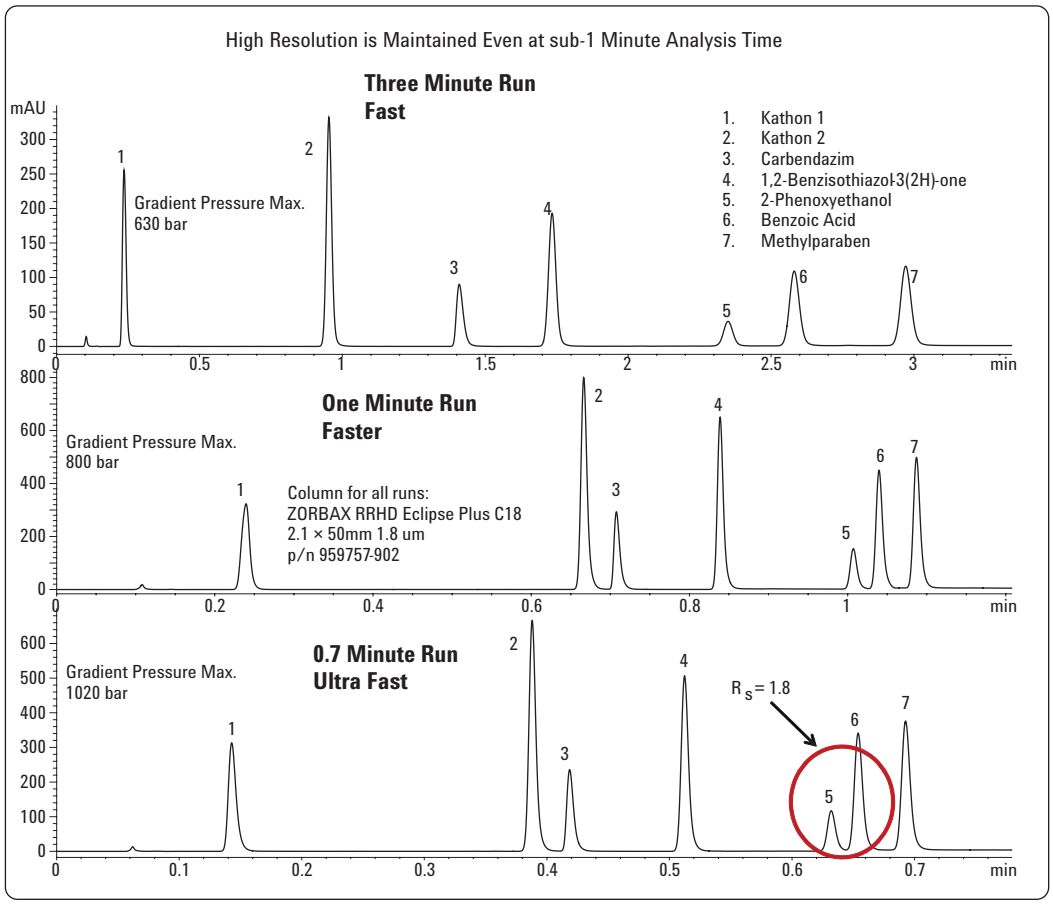


Figure 1. Seven biocides rapidly separated with high resolution using Agilent ZORBAX RRHD Eclipse Plus C18 with three different methods.

Ten consecutive injections of the biocide mix (10 ppm for biocides (1), and (3 to 7) and 30 ppm for biocide (2)) were made using the 1 minute method as shown in Figure 2. The retention time and peak area of each component is listed in Tables 3 and 4 respectively for each of the ten replicate injections. Statistical analysis of the data shows excellent % RSD results that are noteworthy because of the very fast analysis time.

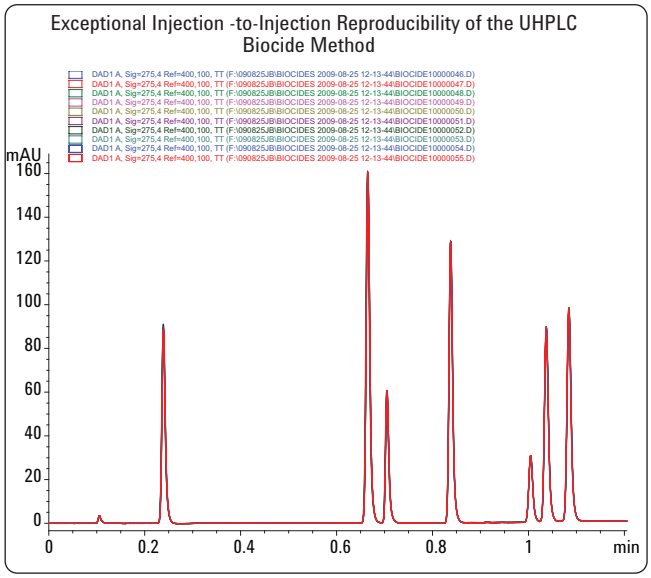


Figure 2. Ten consecutive injections of biocide mix (10 ppm) separated in 1.1 min using a short ZORBAX RRHD Eclipse Plus C18 column.

Table 3. Retention Time Precision Data for Ten Injections of Seven Biocides

	Retention Time (min)						
Injection	**Peak 1**	**Peak 2**	**Peak 3**	**Peak 4**	**Peak 5**	**Peak 6**	**Peak 7**
1	0.239	0.665	0.705	0.838	1.004	1.037	1.084
2	0.239	0.665	0.705	0.838	1.004	1.037	1.084
3	0.239	0.664	0.704	0.838	1.004	1.037	1.084
4	0.24	0.665	0.705	0.838	1.005	1.038	1.085
5	0.239	0.665	0.705	0.838	1.004	1.037	1.084
6	0.239	0.665	0.705	0.838	1.004	1.037	1.084
7	0.239	0.665	0.705	0.838	1.004	1.037	1.084
8	0.24	0.665	0.705	0.838	1.005	1.037	1.084
9	0.239	0.665	0.705	0.838	1.005	1.037	1.084
10	0.239	0.665	0.705	0.838	1.004	1.037	1.084
AVG	0.2392	0.6649	0.7049	0.838	1.0043	1.0371	1.0841
STD	0.00042	0.00032	0.00032	0.00000	0.00048	0.00032	0.00032
%RSD	**0.18**	**0.05**	**0.04**	**0.00**	**0.05**	**0.03**	**0.03**

Table 4. Peak Area Precision Data for Ten Injections of Seven Biocides

	Peak Area						
Injection	**Peak 1**	**Peak 2**	**Peak 3**	**Peak 4**	**Peak 5**	**Peak 6**	**Peak 7**
1	46.116	88.355	30.133	70.249	18.179	53.633	59.922
2	45.971	87.978	30.062	70.054	18.05	53.384	59.666
3	46.266	88.439	30.254	70.467	18.184	53.764	60.053
4	46.115	88.142	30.129	70.311	18.096	53.517	59.879
5	46.164	88.303	30.168	70.544	18.194	53.612	59.934
6	46.286	88.375	30.175	70.655	18.157	53.77	60.046
7	46.19	88.212	30.233	70.292	18.111	53.592	59.85
8	46.178	88.127	30.23	70.416	18.105	53.565	59.932
9	46.158	88.219	30.16	70.441	18.128	53.692	60.054
10	46.165	88.248	30.178	70.342	18.134	53.627	59.949
AVG	46.1609	88.2398	30.1722	70.3771	18.1338	53.6156	59.9285
STD	0.08696	0.13635	0.05724	0.16747	0.04541	0.11481	0.11694
%RSD	**0.19**	**0.15**	**0.19**	**0.24**	**0.25**	**0.21**	**0.20**

Calibration plots of the biocides were used to assess the linearity of the UHPLC method using the 1 minute method. Figure 3 shows an overlay of the seven calibration plots generated by plotting peak area vs. concentration in ppm. Excellent linearity was achieved in the 2.5 to 23 ppm range. Six level calibration plots were done for biocides (1) and (3 to 7) in a concentration range of 2.5 to 20 ppm. A three level calibration plot was done for biocide (2) in a range of 7.5 – 23 ppm.

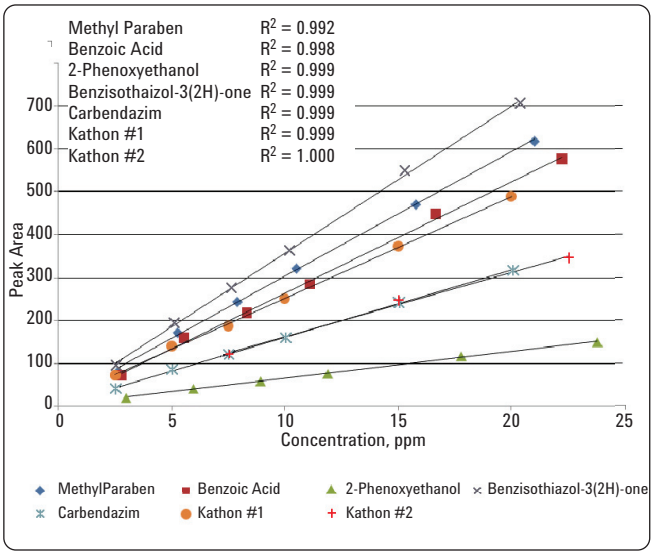


Figure 3. Calibration curves for seven biocides.

A commercial hand sanitizer was analyzed using the ultra fast 0.7 minute method with excellent results. The hand sanitizer was injected neat with no detrimental effects to the column and Figure 4 shows that 2-phenoxyethanol and methylparaben were both easily identified. Methylparaben and 2-phenoxyethanol are common preservatives used in personal care products and were expected to be in this hand sanitizer sample. A number of other trace components were found in the sample, as would be expected from this matrix and none of these peaks interfered with the detection of the biocides.

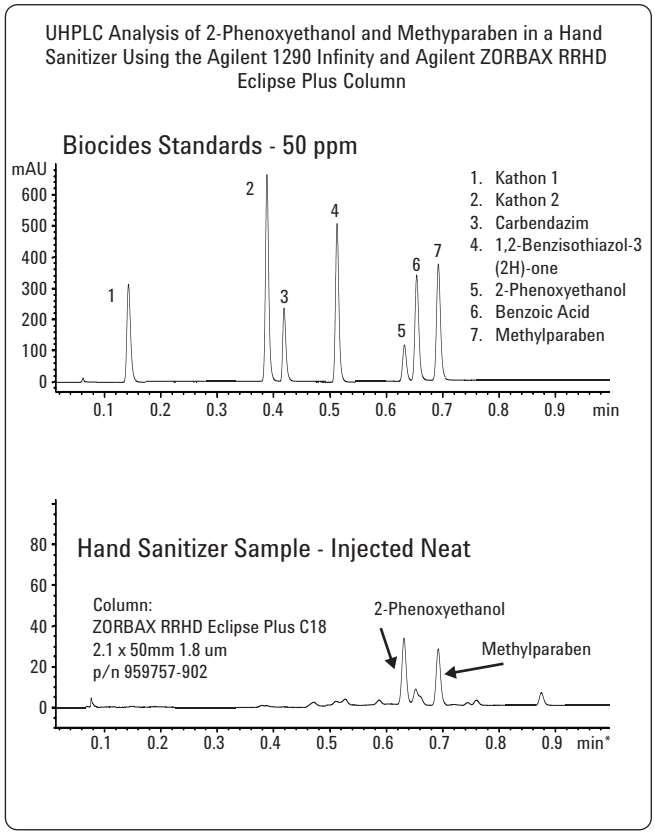


Figure 4. Overlay of biocide standard mix and a hand sanitizer using the sub-1 minute method.

Formic acid (0.1%) can be substituted for TFA for this method. Formic acid is often used in place of TFA when a mass spectrometer (MS) detector is used, to enhance ionization and assist in MS detection. Formic acid was used in the mobile phase to analyze an echinacea supplement using the 1 minute method. The echinacea supplement was diluted 1:5 in 70% methanol and 0.5 µL was analyzed. Methylparaben was easily identified in the sample as shown in Figure 5.

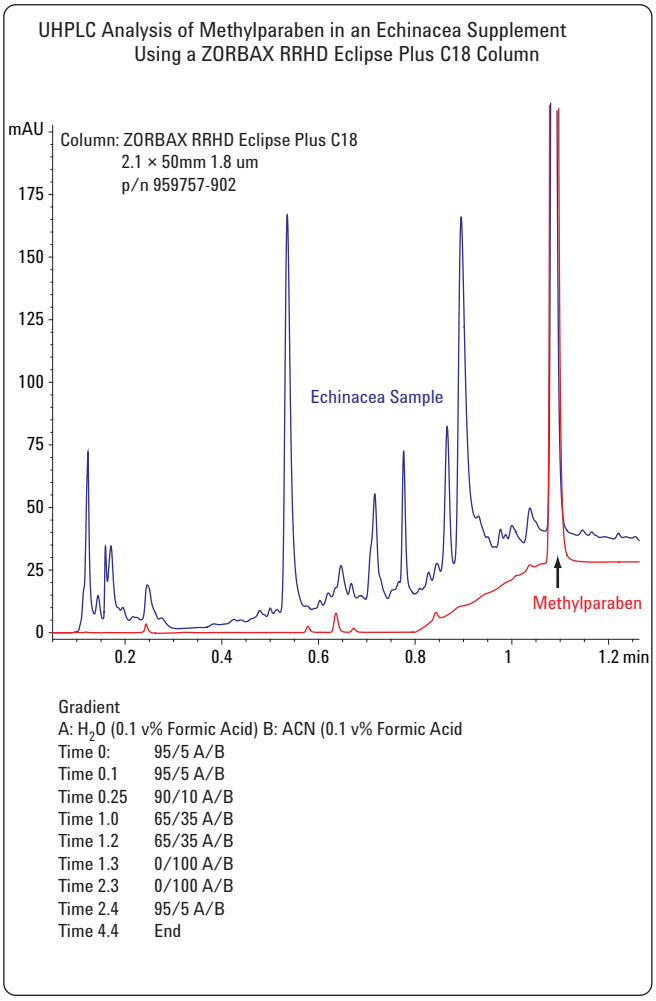


Figure 5. Overlay of methylparaben standard and an echinacea supplement using the 1 minute method with formic acid in the mobile phase.

Conclusion

The Agilent ZORBAX RRHD Eclipse Plus C18 1.8 um column was used in conjunction with the Agilent 1290 Infinity LC System for fast (3 min) to ultrafast (sub-minute), high resolution separation of biocides. The rapid analysis method was successfully applied to the separation and identification of biocides in complex samples such as a hand sanitizer and an echinacea supplement. Benefits include speed with high resolution for improved productivity and reproducibility.

References

1. Derek J. Knight and Mel Cooke, "The Biocide Business: Regulation, Safety and Applications," Wiley-VCH 2002.

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