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Application Note

ACQUITY UPLC PDA Analysis of Biocides (Part 1)

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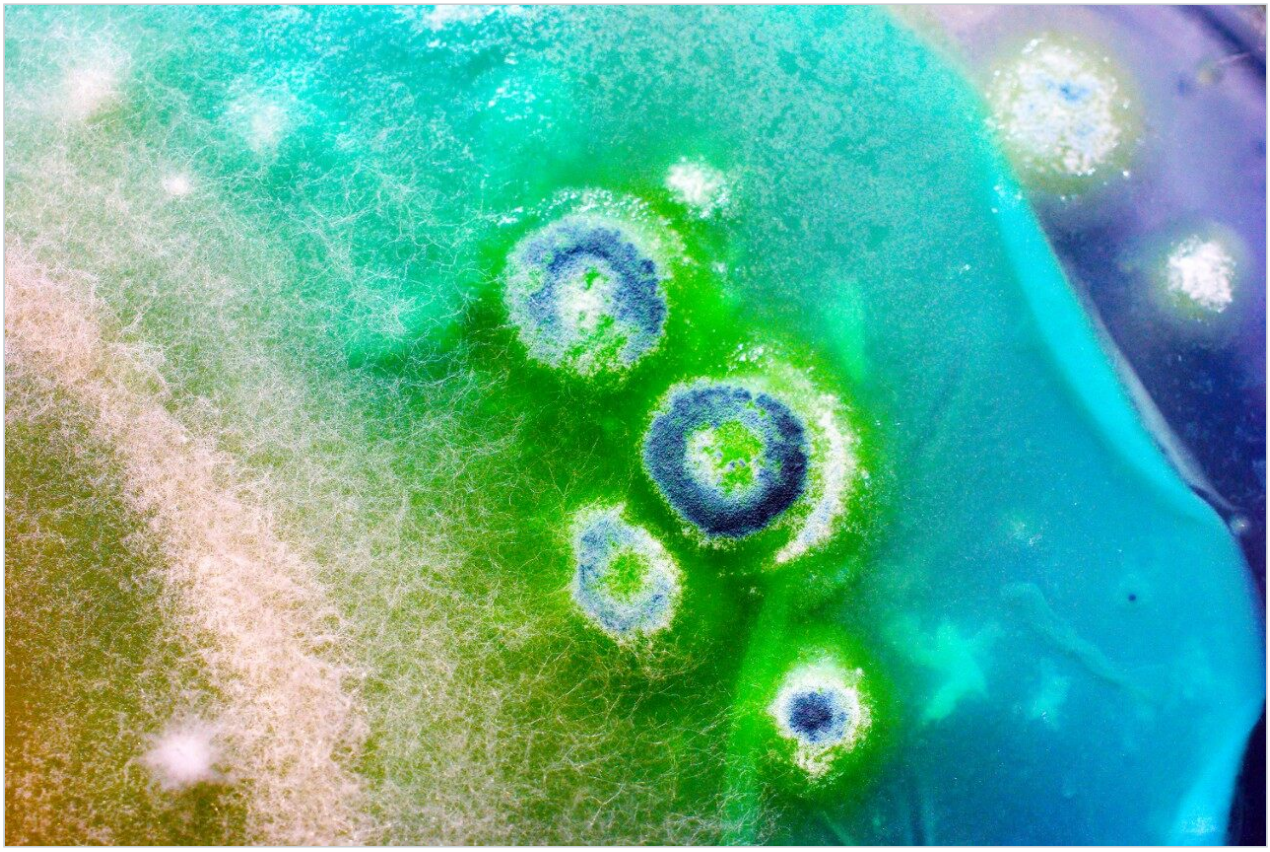

Abstract

This application note describes a three-minute separation of six biocides using the Waters ACQUITY UPLC PDA System with Empower 3 Software.

Benefits

- Improves laboratory productivity by enabling the rapid and sensitive separation of six commonly used biocides.
- Library matching and quantification automated with Empower 3 Software increases confidence in peak confirmation and helps ensure product quality.
- Suitable for cosmetic and personal care product development and quality control analytical testing.

Introduction

Unwanted micro-organisms such as bacteria, viruses, and molds can grow wherever there is a source of nutrition and moisture. This unwanted growth may negatively impact human health, interfere with manufacturing processes, damage building structures, and spoil consumer goods. The principal defense against deleterious micro-organisms is biocides, commonly classified as disinfectants, preservatives, antifouling products, and pest controls.

Biocides are used as additives in cosmetics and personal care, household, and industrial products. To protect the environment and human health, many countries regulate biocide use.[1] In the European Union, this is done through the Directive 98/8/EC (The Biocidal Products Directive) and Regulation (EU) No 528/2012 (The Biocidal Products Regulation). In the United States, regulatory control of biocides falls under the EPA and the biocides applications in cosmetics, food, and personal health care are regulated by the U.S. FDA. Regulations impact the registration, labeling, and composition of biocides.[2] Reliable and rapid methods are therefore essential to ensure effective product quality control. This application note describes a three-minute separation of six biocides using the Waters ACQUITY UPLC PDA System with Empower 3 Software. With PDA library matching and the built-in advanced mathematical algorithms, each biocide in the mixture can be identified and quantified; the analysis is fast and reproducible. The ability to quickly and unambiguously analyze biocide content can facilitate workflow related to the quality control and regulatory compliance of biocide containing products. This methodology benefits new product development, product troubleshooting and biocide production.

Experimental

Sample preparation

Analytes are:

Kathon CG/ICP [containing 0.4% of 2-methyl-4-isothiazolin-3-one (1a), 1.2% of 5-chloro-2-methyl-4-isothiazolin-3-one (1b)];

Carbendazim (2);

Benzisothiazol-3(2H)-one (3);

2-phenoxyethanol (4);

Benzoic Acid (5);

Methyl paraben (6).

LC conditions

LC system:	ACQUITY UPLC PDA
Software:	Empower 3
Weak wash:	95:5 Water: CH_3CN (600 µL)
Strong wash:	50:50 Water:CH_3CN (200 µL)
Seal wash:	90:10 Water: CH_3CN (5 min)
Column temp.:	30 °C
Flow rate:	1 mL/min
Injection:	5 µL
Detection:	PDA 210 to 500 nm

Sampling rate:	20 pts/s
Filter response:	0.1 s
Column:	ACQUITY UPLC BEH C_{18} 2.1x 50 mm
Mobile phase A:	0.05 v% Trifluoroacetic acid (TFA) in water
Mobile phase B:	0.05 v% TFA in CH_3CN
Linear gradient:	5% to 15% B in 2.9 min

Note: The column was equilibrated with 5% B for 2 minutes before injection, and was washed with 100% B for 2 minutes at the end of each run.

Results and Discussion

Figure 1 shows the structures of the biocides (1a, 1b, 2-6); a 5 ppm mixture of 1–6 was separated using the Waters ACQUITY UPLC System with a three-minute linear gradient method. These compounds are frequently used in adhesives, paint and coatings, latex and sealants, inks, wood and paper products, textile and leather products, metalworking fluids, personal care products, cosmetics, laundry detergents, dishwashing liquids, and household and industrial cleaners. The acetonitrile/ water mobile phase with TFA modifier is compatible with mass spectrometry detectors, if needed.

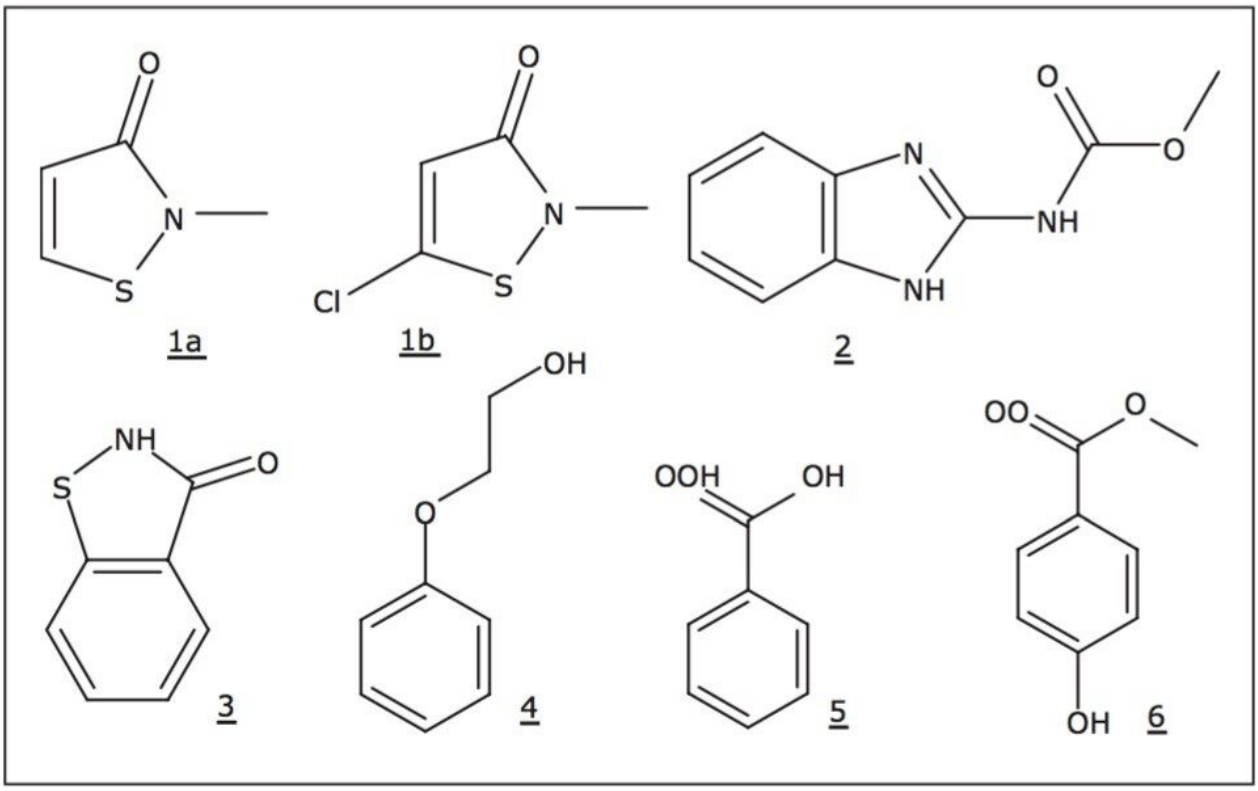

Figure 1. Chemical structures of biocides.

UV photodiode array (PDA) detection combined with Empower 3 Software enables a powerful range of detection and identity confirmation possibilities for chromatographic separations. PDA timed wavelength chromatograms can be plotted using the λmax of each analyte. This increases the detection limit when the analytes have very different λmax and aids quantification. Figure 2 is an overlay of nine replicate injections of PDA timed wavelength chromatograms, demonstrating that the overall reproducibilty is excellent. The three-minute linear gradient easily resolves the two active components of Kathon CG/ICP (1a and 1b) and the other five biocides. The retention time and peak area of each component observed in the above nine replicate injections are listed in Tables 1 and 2, with statistical analysis results generated using Empower 3 Software. The excellent % RSD results indicate the suitability of UPLC with BEH column chemistry for biocides.

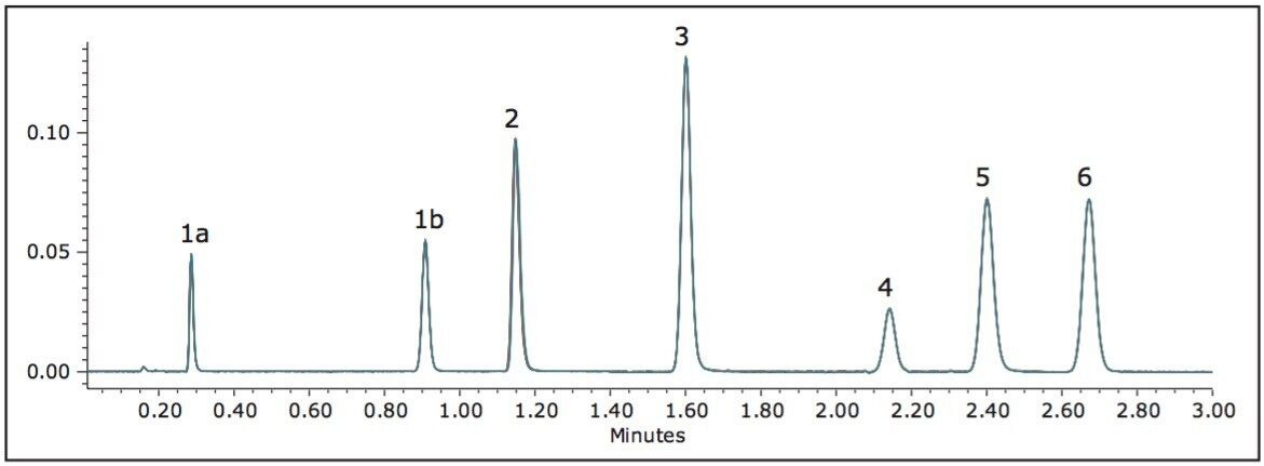

Figure 2. Overlay PDA timed wavelength chromatograms, retention time and peak area tables of 9 replicate injections of sample containing 1.25 ppm of 1a, 3.75 ppm of 1b and 5 ppm of 2–6: (0.00 min, 275 nm; 1.40 min, 225 nm; 2.55 min, 255 nm).

	1a (min)	1b (min)	2 (min)	3 (min)	4 (min)	5 (min)	6 (min)
1	0.286	0.908	1.147	1.600	2.141	2.400	2.671
2	0.287	0.908	1.147	1.601	2.141	2.400	2.671
3	0.286	0.908	1.147	1.601	2.141	2.401	2.672
4	0.286	0.908	1.148	1.602	2.142	2.401	2.672
5	0.286	0.908	1.148	1.601	2.141	2.400	2.671
6	0.286	0.908	1.149	1.602	2.142	2.401	2.672
7	0.287	0.908	1.148	1.601	2.141	2.400	2.671
8	0.286	0.908	1.150	1.602	2.142	2.401	2.672
9	0.287	0.909	1.150	1.602	2.142	2.401	2.672
Mean	0.286	0.908	1.148	1.601	2.142	2.401	2.672
Std. Dev.	0.001	0.000	0.001	0.001	0.001	0.001	0.001
% RSD	0.19	0.04	0.10	0.03	0.03	0.02	0.02

Table 1. Component summary for retention time for 9 replicate injections of sample containing 1.25 ppm of 1a, 3.75 ppm of 1b and 5 ppm of 2–6: (0.00 min, 275 nm; 1.40 min, 225 nm; 2.55 min, 255 nm).

	1a	1b	2	3	4	5	6
1	34745	68748	134846	227719	57857	172510	173458
2	34684	69423	134511	227840	57682	170783	172192
3	34730	68894	134698	228692	57882	173440	172053
4	34741	69168	135187	228238	57388	173125	172113
5	34761	68952	134533	228331	58008	170433	172156
6	34673	69132	134817	228461	57802	170579	171725
7	34753	68903	135014	228616	57863	172557	171723
8	34781	68736	135018	227710	57845	170954	171833
9	34782	69050	134694	228489	57809	172072	172143
Mean	34739	69001	134813	228233	57793	171828	172155
Std. Dev.	38	219	229	383	174	1157	523
% RSD	0.1	0.3	0.2	0.2	0.3	0.7	0.3

Table 2. Component summary for area for 9 replicate injections of sample containing 1.25 ppm of 1a, 3.75 ppm of 1b and 5 ppm of 2–6: (0.00 min, 275 nm; 1.40 min, 225 nm; 2.55 min, 255 nm).

Six levels of calibration standards (containing Kathon and 2–6 from 2.5 to 20 ppm) were analyzed. Empower 3 Software has built-in mathematical features and functions. Calibration curves were created from the standards and the quantity of analyte in each sample was calculated automatically. Figure 3 shows the calibration plots generated by Empower 3, using the peak areas vs the concentration. The linearity of the calibration curves is excellent with the R^2 values (residual sum of squares) above 0.9999, except one with 0.9998. Table 3 shows a typical results analysis table for peak identification and quantification using a biocides standard mixture mix as a sample. The last column shows that amounts match well with actual values (1.25 ppm for 1a, 3.75 ppm for 1b, and 5 ppm for 2–6). The data suggest that UPLC/PDA is well suited to meet the regulatory demands for quantitative analysis of biocides.

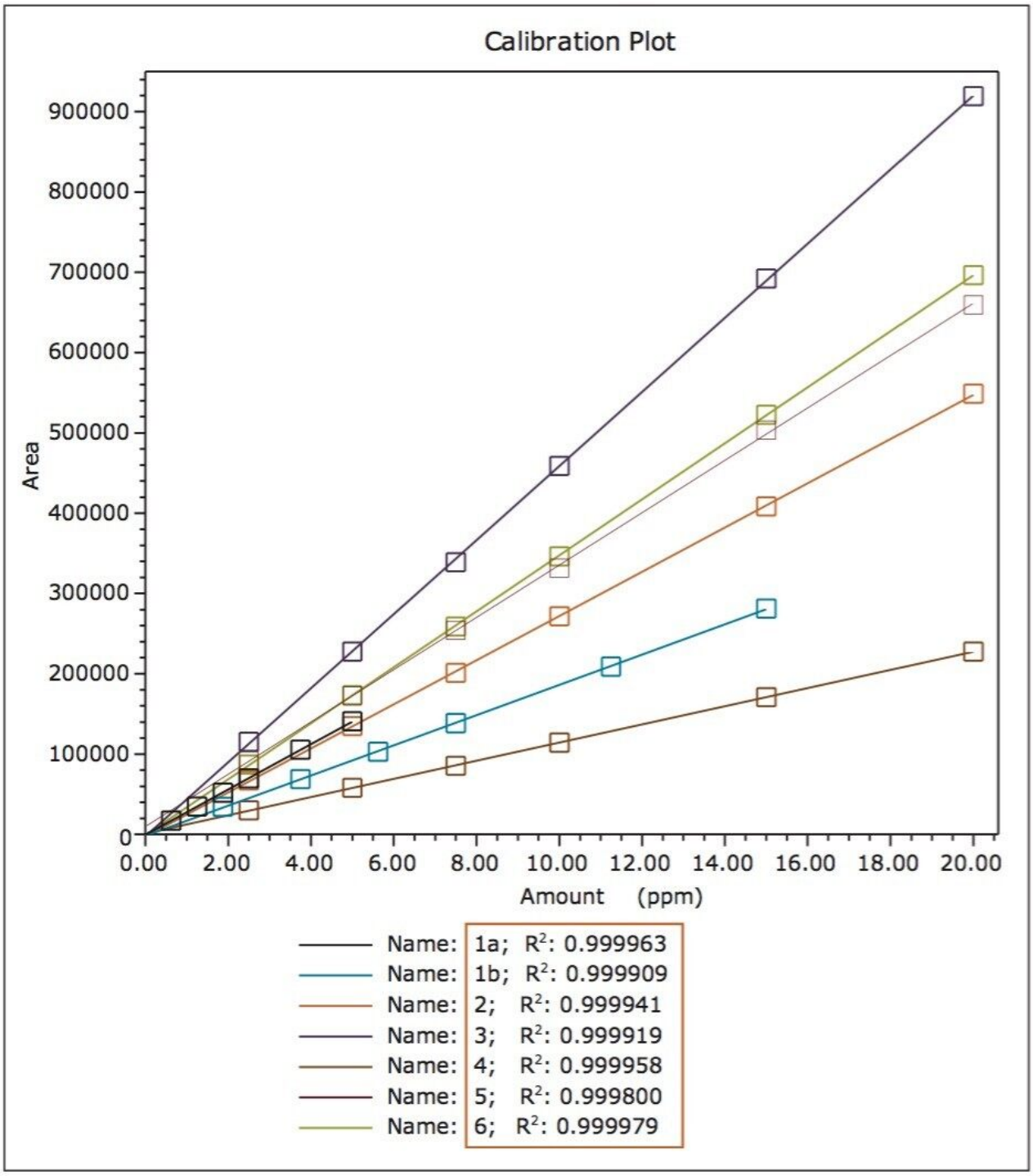

Figure 3. Calibration curves for (1a, 1b, 2-6).

Empower 3 Software provides the capability of creating a PDA library from pure component peaks in user chromatograms. Afterwards, the library matching and peak purity features can be used with samples to confirm peak identities and to give added confidence that spectrally distinct peaks are not-coeluting.

Empower 3 uses Spectral Contrast theory to quantitatively compare the shapes of UV spectra during library matching and Peak Purity analysis.[3-6] Figure 4 shows UV spectra, extracted from PDA chromatograms of standards (1a, 1b, 2-6); these spectra were used to create a library with names and retention times. Table 3 shows an example of a default Empower Report table with PDA library matching and Peak Purity results. The values of Match Angle are smaller than Match Threshold and the values of Purity Angle are smaller than Purity Threshold, indicating that the analytes were well separated and matched with PDA library of biocides.

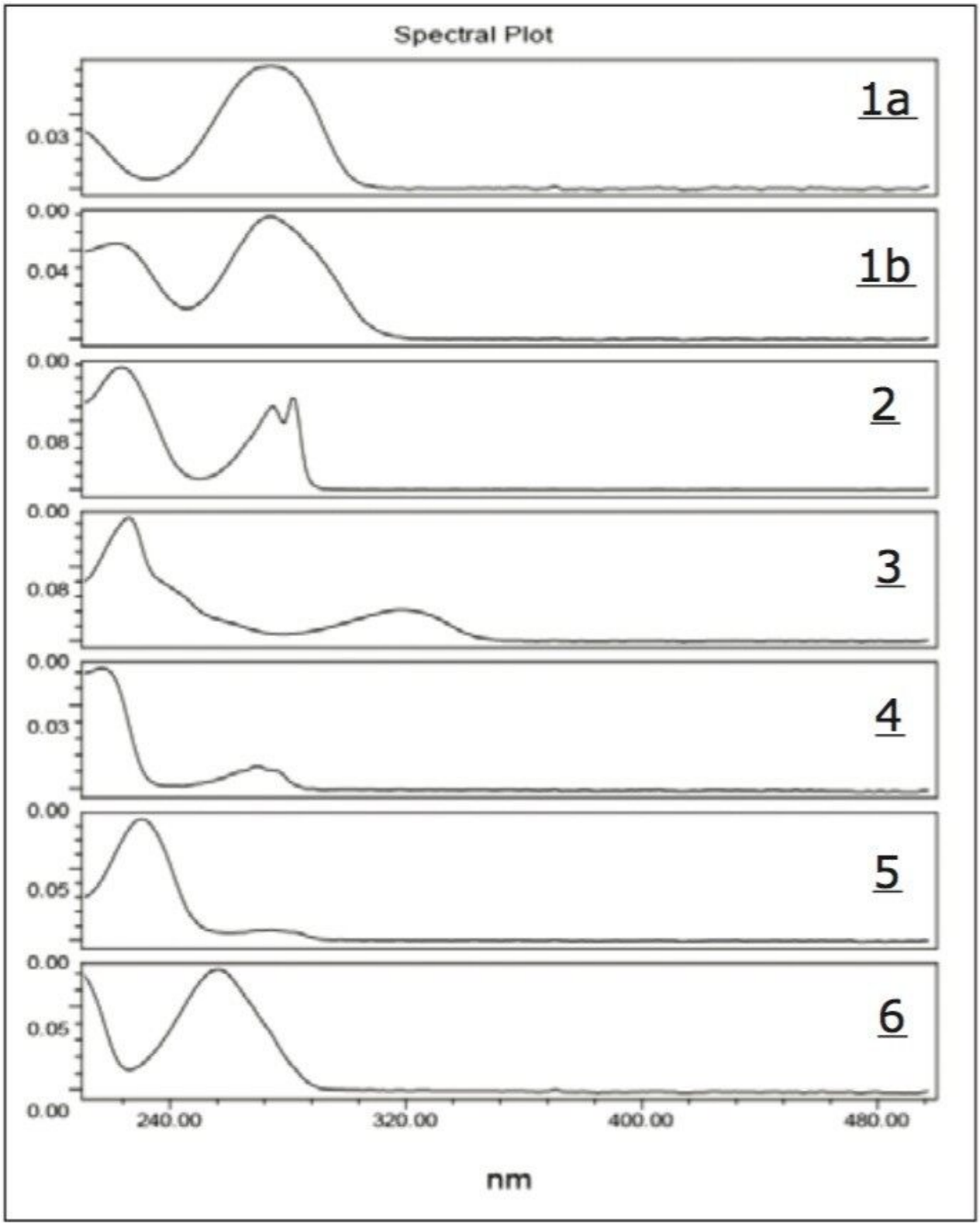

Figure 4. UV spectra of 1a, 1b, and 2-6 extracted from PDA data.

	Component	Match1 Spect. Name	Match1 Angle	Match1 Threshold	Purity1 Angle	Purity1 Threshold	Amount (ppm)
1	1a	2-methy l-4-isothiazolin-3-one	0.312	1.598	0.792	1.086	1.25
2	1b	5-chloro-2-methy l-4-isothiazoline-3-one	0.401	1.474	0.676	0.863	3.78
3	2	carbendazim	0.184	1.244	0.337	0.554	5.01
4	3	1,2-benzisothiazol-3-one	0.174	1.282	0.368	0.628	5.01
5	4	2-phenoxyethanol	0.992	2.663	2.296	2.461	5.00
6	5	benzoic acid	0.322	1.399	0.560	0.760	4.99
7	6	methyl paraben	0.235	1.388	0.508	0.743	4.97

Table 3. Peak identification and quantification results shown on an Empower Report table, with additional PDA library matching and Peak Purity results.

Conclusion

Compliance with regulations that limit the type and concentration of biocides in a variety of applications necessitates analytical testing. This note illustrates that the Waters ACQUITY UPLC System with PDA detection enables rapid and sensitive separations of six commonly used biocides. With Empower 3 Software, library matching and quantification can be automated to add confidence in peak confirmation that is unavailabe with a single wavelength UV detector. This method is simple to use and suitable for quality control, new product development, and troubleshooting for both cosmetic and personal care manufacturers.

References

1. D J Knight, M Cooke. *The Biocides Business Regulation, Safety and Applications*, WILEY-VCH 2002,ISBN 3-527-30366-9.
2. United States Environmental Protection Agency. Antimicrobial Pesticide Registration. [cited 2015 November 9]. Available from: http://www2.epa.gov/pesticide-registration/antimicrobial-pesticide-registration.
3. M V Gorenstein, et al. Detecting Co-eluted Impurities by Spectral Comparison. *LCGC*. 12 (10): 768–772, 1994.

4. Peak Identification with Waters 996 Photodiode Detector: Library Matching, Waters Corporation, WPP12, Library No 960405.

5. Empower\Help\Interpreting PDA Peak Purity Results.

6. P J Lee, A J Di Gioia, ACQUITY UPLC/PDA: UV Filter Agents and Preservatives, Waters application note no. 720001770, 2006.

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