APPLICATION NOTE

UHPLC

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Analysis of Biocides with the PerkinElmer Flexar FX-15 UHPLC System Equipped with a PDA Detector

Introduction

Biocides are chemical substances that are used to kill or inhibit harmful organisms. Biocides have a wide range of applications in consumer and industrial products. In the food industry methylparaben is used as a preservative, and benzoic acid and its salts are widely used in beverages to prevent the growth of mold and yeast. In cosmetic products, methylisothiazoline is used to prevent the growth of some microbes. In agriculture, carbendazim is used as a fungicide and is effective in treating fungal diseases of trees. In construction, benzisothiazoline is used as an antimicrobial and fungicide in adhesive and varnishes.

While undoubtedly biocides have useful applications, their uses need to be regulated because they can be very toxic to humans. In vitro studies have shown that methylisothiazoline is neuro-toxic and can cause damage to rat brain cell in tissues culture. There are mounting evidences that support the suspicions that carbendazim exposure causes birth abnormalities in humans and other animals: in 1990, a pregnant Florida woman exposed to benlate (which breaks down into carbendazim) through her occupation later gave birth to a child without eyes. In recent years, there has been considerable concern that the indiscriminate use of biocides increases bacteria resistance, which can cause previously treatable diseases to be become untreatable.

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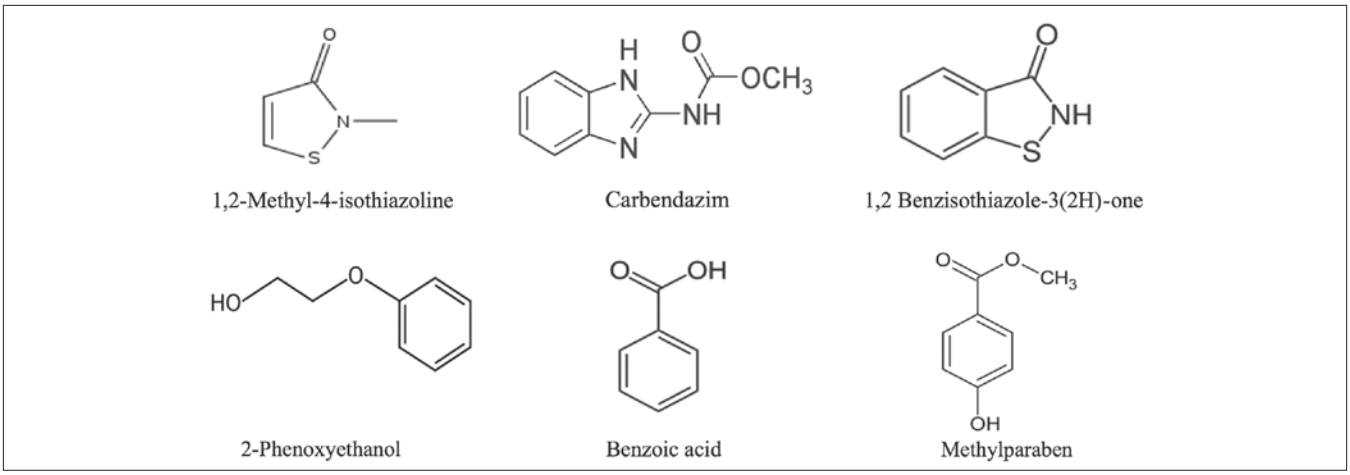

Figure 1. Molecular structure of biocides studied.

In the U.S., biocides are subdivided in categories that are regulated by different agencies. Personal health care products and disinfectants are regulated by the FDA, and the Cosmetic Act (FD&C Act). However, regardless of the regulatory agency, it is against the law to market products with ingredients that may cause injuries under condition of use recommended in labeling. It is therefore the responsibility of industries to use biocides at levels that are safe. To that end, there is a need for analytical methods to constantly monitor the type and amount and comply with regulations.

This application note presents a robust and sensitive reverse phase liquid chromatography method for the analysis of six widely used biocides (Figure 1). Method conditions and performance data including precision and linearity are presented. A widely used antimicrobial hand soap is analyzed and the biocides type and concentration are confirmed.

Experimental

A stock standard with 0.6 mg/mL of methylisothiazoline and methylparaben, and 1.2 mg/mL of the four other biocides were prepared by transfer of each of the net weight into the same 10 mL volumetric flask; 1.0 mL of isopropanol was added and the solution was brought to volume with a solution of 1:1 methanol:water and then sonicated for five minutes. 1.0 mL of the stock standard was transferred into a 10 mL volumetric flask and brought to volume with water. The solution obtained had 0.06 mg/mL of methylisothiazoline and methylparaben, and 0.12 mg/mL of each of the other biocides.

Precision was evaluated with six injections of the working standard. Linearity was determined across a range of 0.2 to 120 µg/mL concentration. About 1 g/mL of a popular antibacterial hand soap was prepared by dilution with water. Samples were thoroughly mixed and filtered through a 0.2 µm nylon membrane prior to testing.

A PerkinElmer® Flexar® FX-15 UHPLC system fitted with a Flexar FX PDA photodiode array detector was the platform for this experiment. The separation was achieved using a Superficially Porous Particle Column (Brownlee SPP C18, 2.7 µm, 50 x 2.1 mm). The run time was 3.5 minutes with a back pressure of 4300 PSI (297 bar).

Results And Discussion

Initially, the method was developed with a conventional C18, 250 x 4.6 mm, 5 µm particle size HPLC column. The optimal flow rate of this method was determined to be 1.0 mL/min. at 40 °C. All the peaks eluted within 16 minutes (see Figure 2). By using a shorter column with smaller particle size (C18 50 x 2.1 mm, 2.7 µm particle size) suitable for UHPLC, the run time was dramatically reduced from 16 minutes to about 3.5 minutes at 40 °C and the resolution, and sensitivity was significantly improved. Prior to running the sample, from one injection of the standard the maximum wavelength for each peak was determined and the wavelength recording setting was optimized accordingly (see Figures 3 and 4).

Table 1. **Detailed UHPLC system and chromatographic conditions.**

Autosampler:	Flexar FX UHPLC
Setting:	50 µL Loop and 15 µL needle volume, partial loop mode, 350 µL mixer volume
Injection:	10 µ, 2 µL; injector wash:water
PDA Detector:	Scanned from 190 – 700 nm, recording setting 255, 225 and 257 nm
HPLC Column:	PerkinElmer Brownlee™ Analytical C-18, 5 µm, 250 x 4.6 mm Part No. N9303514 at 40 °C
Mobile Phase:	B: 0.05% TFA in 60:40 Methanol:Acetonitrile A: 0.05% TFA in water

Time (min.)	Flow rate (mL/min.)	B %	Curve
10	1.0	15-40	1
8	1.0	40	1

2-3 minutes equilibration after each injection (HPLC grade solvent and ACS grade reagent)

UHPLC Column:	PerkinElmer Brownlee SPP C-18, 50 x 2.1 mm, 2.7 µm (Superficially Porous Particle) Part No. N9308402 at 40 °C
Mobile Phase:	B: 0.05% TFA in Acetonitrile A: 0.05% TFA in water

Time (min.)	Flow rate (mL/min.)	B %	Curve
3.5	0.4	15-24	1

3 minutes equilibration after each injection

Time (min.)	Wavelength (nm)
0-0.5	275
0.5-3	225
3-3.5	257

(HPLC grade solvent and ACS grade reagent)

Software:	Chromera Version 3.0
Sampling Rate:	5 pts/s

PerkinElmer's Chromera® software allowed the creation of a spectral library (Figure 6), that was later used for peak identification confirmation in the sample (Figure 7).

In addition to the more than fourfold reduction in chromatographic run time, the flow rate was reduced to 0.4 mL/min from 1.0 mL/min. Thus, 78% reduction in testing time and 91% reduction in solvent usage was achieved by moving to the UHPLC method. This is important not only because of the relatively high cost of HPLC-grade solvents, but also because far less solvent are disposed of as waste. This results in a much lower cost of ownership and a much greener laboratory operation.

Excellent method performance was achieved. The linearity of the analysis achieved an R-squared value ranging from 0.9998 to 1 and precisions values ranged from 0.6 – 0.8% RSD. Resolution between consecutive peaks was outstanding with value of 3 or more. Details of the method performance and results of the sample tested are presented in Table 2.

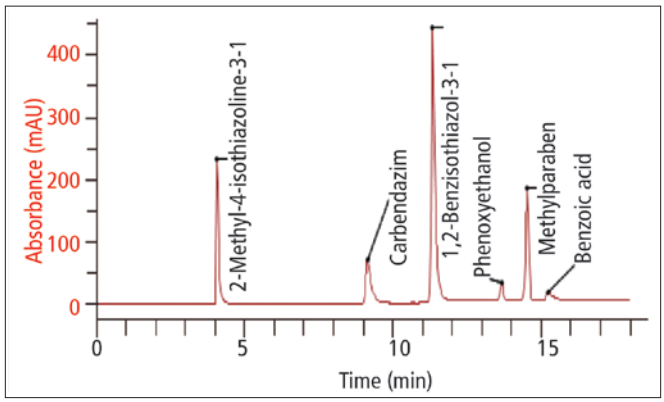

Figure 2. Chromatogram from the analysis of the standards using a conventional HPLC C18 250 x 4.6 mm, 5 µm column.

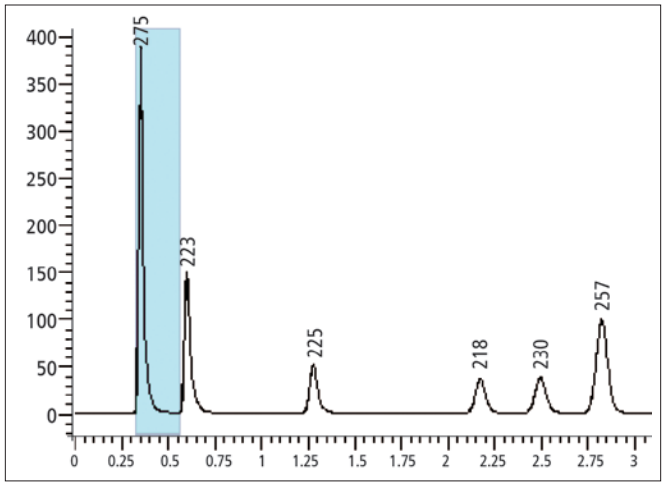

Figure 3. Chromatogram from the analysis of the standards showing the maximum absorbance for each peak.

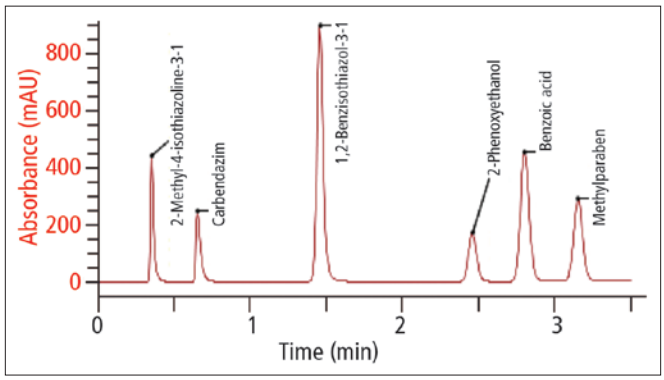

Figure 4. Chromatogram from the analysis of the standards using a UHPLC C18 50 x 2.1 mm, 2.7 µm column.

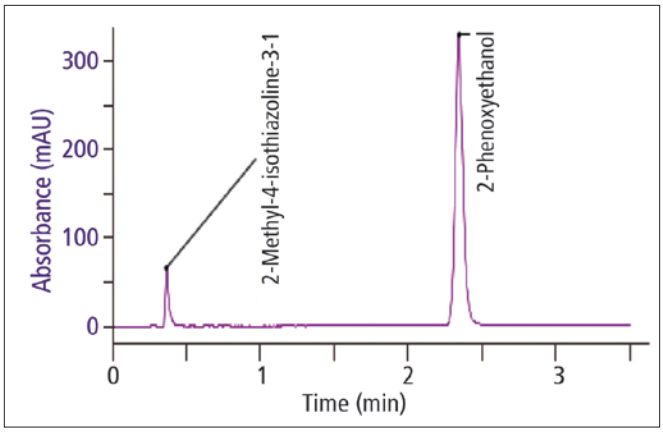

Figure 5. Separation of biocides in a popular antibacterial hand soap.

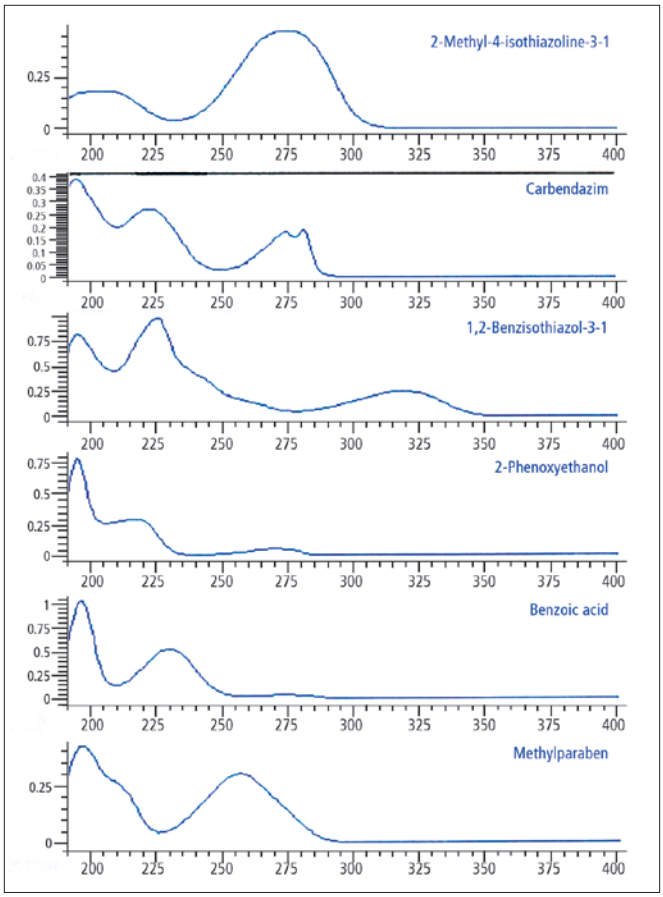

Figure 6. UV Spectra from the standards.

Conclusion

The application of UHPLC to the analysis of biocide resulted in a fourfold reduction in run time, as well as 91% reduction in solvent usage. The PerkinElmer Flexar FX-15 UHPLC system and PerkinElmer Brownlee SPP C-18, 2.7 µm, 50 x 2.1 mm resolved all the six biocides with a resolution ranging from 3 to 12 between consecutive peaks. The method was shown to be linear with a good precision. The antibacterial hand soap tested has 0.01% (~100 ppm) methylisothiazoline, and 0.25% (~2500 ppm) phenoxyethanol, well within the respective limit of up to 100 ppm and 10,000 ppm recommended by the U.S. Cosmetic Ingredient Review expert panel, and the EU Cosmetic Directive.

PerkinElmer's FX PDA detector provides rugged and accurate detection over a range of 190 nm to 700 nm, encompassing UV and visible wavelengths. The Chromera software offers many data acquisition and processing features: spectral library creation, and peak purity, spectra 3D and contour maps, which are powerful tools for interrogating the information content of a 3D photodiode array chromatogram. The spectra library search function allowed the storage of standard peaks spectra that were later used for peak identification confirmation in the sample.

Table 2. **Precision, linearity and amount in sample.**

	%RSD (n=6)	Resolution	Linearity r^2	Range (µg/mL)	Hand Soap %
1,2-Methyl-4-isothiazoline	0.7	NA	0.9998	60 - 0.2	0.01
Carbendazim	0.7	6.7	1	120 - 0.4	ND
1,2 Benzisothiasole-3(2H)-one	0.6	12.6	1	120 - 0.4	ND
2-Phenoxyethanol	0.6	11.4	1	120 - 0.4	0.25
Benzoic acid	0.7	3.3	1	120 - 0.4	ND
Methylparaben	0.8	3.3	1	60 - 0.2	ND

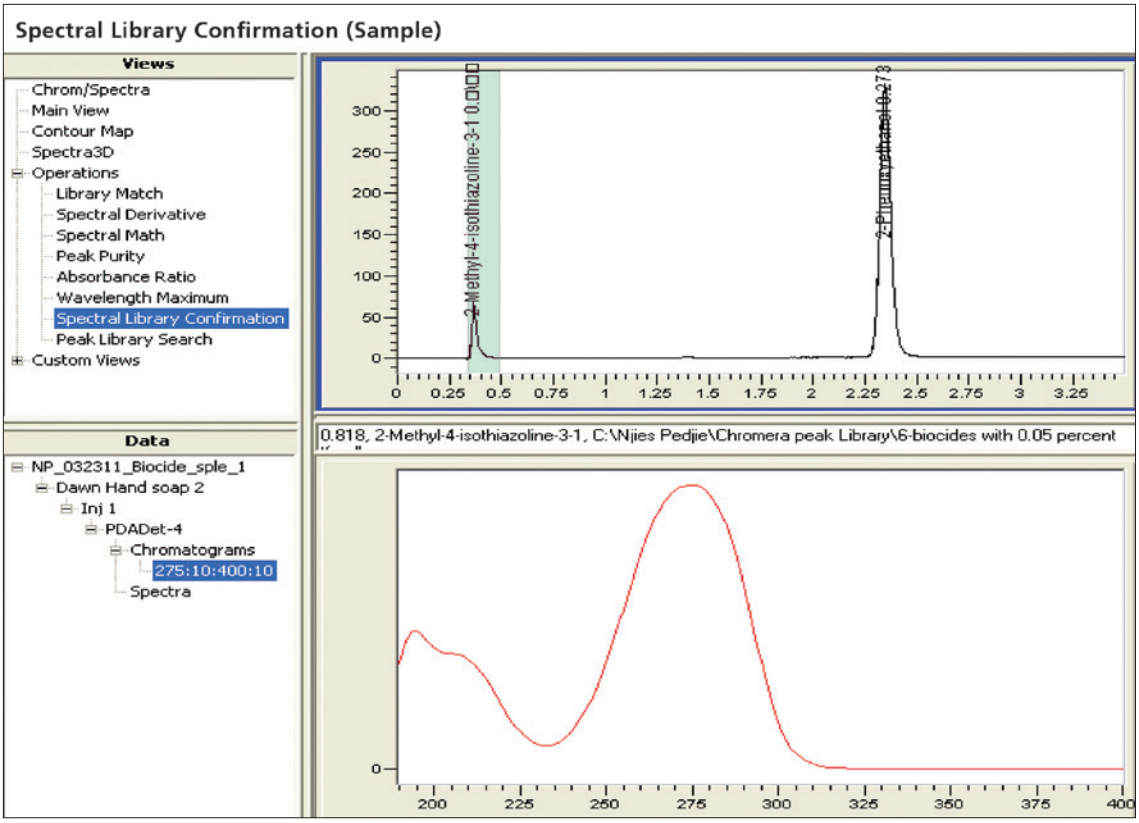

Figure 7. Peak identification using the spectral library.

References

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Note: This application is subject to change without prior notice.

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