



# Analysis of Biocides Using Compact HPLC

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## Abstract

The Axcend Focus LC® (a compact capillary liquid chromatograph) has been successfully used for quantitative analysis of two representative biocides: 4,5-dichloro-2-n-octylisothiazolin-3-one (DCOIT) in wood extracts and 4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl)-1H-pyrrole-3-carbonitrile (ECONEA®) in sea water.



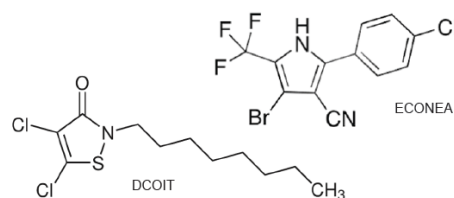
## Introduction

Biocides are widely used to suppress undesired organisms such as insects, rodents, bacteria, and fungi. Since biocides can be toxic to humans and unintended animals besides the target organism(s), they must be applied at strictly controlled concentrations.

4,5-Dichloro-2-n-octylisothiazolin-3-one (DCOIT) is a fungicide used to protect wood from decay. An example is its use to prolong the life of utility poles. After the wood is treated, the correct content of the applied biocide must be confirmed.

4-Bromo-2-(4-chlorophenyl)-5-(trifluoromethyl)-1H-pyrrole-3-carbonitrile (ECONEA) is used in anti-fouling coatings for various underwater surfaces to protect them against algae and mollusks. Since ECONEA potentially can be released from the coating, water samples must be tested for leached biocide.

The chemical structures of DCOIT and ECONEA are shown in Figure 1.



**Figure 1.** Chemical structures of 4,5-dichloro-2-n-octylisothiazolin-3-one (DCOIT) and 4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl)-1H-pyrrole-3-carbonitrile (ECONEA).

Since the compact Focus LC is portable and can operate with battery power, it is a convenient and powerful tool for on-site analysis of target analytes. As demonstrated here, this instrument can be successfully used for quantitative analysis of DCOIT in wood extracts and ECONEA in simulated ocean water samples.

## Experimental Conditions

### Instrumentation

An Axcend Focus LC with 3.1.0 Axcend Drive™ software was used for all measurements.

### Test Compounds and Solvents

HPLC grade mobile phase solvents (acetonitrile, methanol, water, and trifluoroacetic acid) were purchased from Sigma-Aldrich (MilliporeSigma, St. Louis, MO, USA). Simulated sea water was prepared using sea salt from Instant Ocean (Spectrum Brands, Blacksburg, VA, USA).

### HPLC Column and UV-absorption Detection

For the analysis of DCOIT and ECONEA, an Acquity HSS T3 column was used (10 cm x 150 µm i.d., 1.8 µm particle size) from Waters (Milford, MA, USA). The separations were monitored by UV-absorption at 275 nm using an Axcend capillary flow-cell detector.

### Chromatographic Methods

DCOIT was analyzed using 2% methanol in water as Solvent A and acetonitrile as Solvent B. The mobile phase composition at a flow rate of 1 µL/min was: 70% B (0-11.5 min), 70-99% B (11.5-12 min), and 99% B (12-15 min). Timed injections were used with the injection valve in the “Inject” position for 0.01 min, which resulted in an injection volume of 10 nL.

For the analysis of ECONEA, Solvent A contained 97% water, 3% acetonitrile, and 0.1% trifluoroacetic acid; and Solvent B was comprised of 97% acetonitrile, 3% water, and 0.1% trifluoroacetic acid. In this case, the mobile phase gradient at 1 µL/min was:

3% B (0-6.1 min), 3-97% B (6.1-12 min), and 97% B (12-17 min). An injection volume of 6 µL was provided by using a 6 µL external injection loop with an injection time of 6 min.

All mobile phases and samples were filtered through Kinesis KX regenerated cellulose (hydrophilic) or PTFE (hydrophobic) syringe filters with 0.22 µm pore size (Cole-Parmer, Vernon Hills, IL, USA).

## Results and Discussion

### Quantitative Determination of DCOIT in Wood Extracts

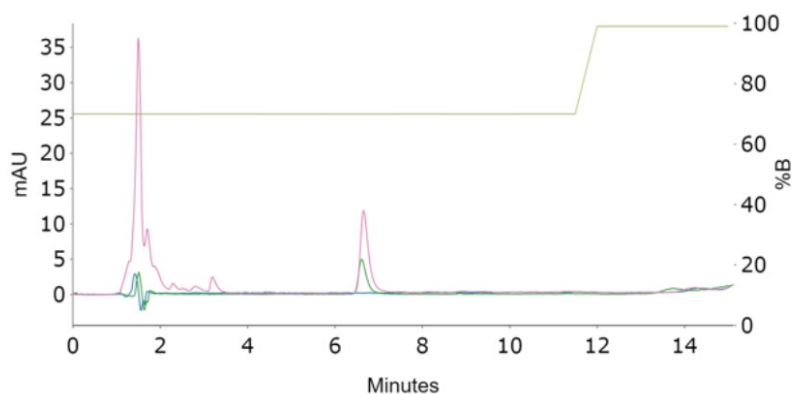
The concentration of DCOIT in wood extracts was determined using a two-point calibration curve with five replicate runs of calibration standard. Representative chromatograms are shown in Figure 2. The results for the calibration standards and test sample are summarized in Tables 1 and 2, respectively. The signal-to-noise ratio at the lower concentration of 20 µg/mL (70.9 µM) was 26 or higher, suggesting that this concentration is substantially above the

lower limit of quantification. At a concentration of 80 µg/mL (283 µM), the peak height did not exceed 20 mAU. This is well within the linear range of the UV-absorption detector. Based on these results, the calibration range can be extended, if necessary, to lower and/or higher concentrations.

DCOIT 20 µg/mL (70.9 µM)			DCOIT 80 µg/mL (283 µM)		
Retention time, min	Peak height, mAU	Peak area, mAU*s	Retention time, min	Peak height, mAU	Peak area, mAU*s
6.59	4.90	64.3	6.59	16.8*	218*
6.52	4.93	63.9	6.57	19.2	249
6.56	4.91	63.6	6.60	19.5	254
6.55	4.93	65.4	6.60	19.6	252
6.58	4.92	64.4	6.58	19.8	256
Average	4.92	64.3	Average	19.5	253
Std Dev	0.0149	0.663	Std Dev	0.256	3.34
RSD, %	0.304	1.03	RSD, %	1.31	1.32

\*Excluded as an outlier based on Grubbs' test.

**Table 1.** Analysis of DCOIT Calibration Standards.



**Figure 2.** Overlaid chromatograms of a blank sample (methanol, blue trace), a DCOIT calibration standard at 20 µg/mL (green trace) and a methanolic extract of wood pre-treated with DCOIT (pink trace).

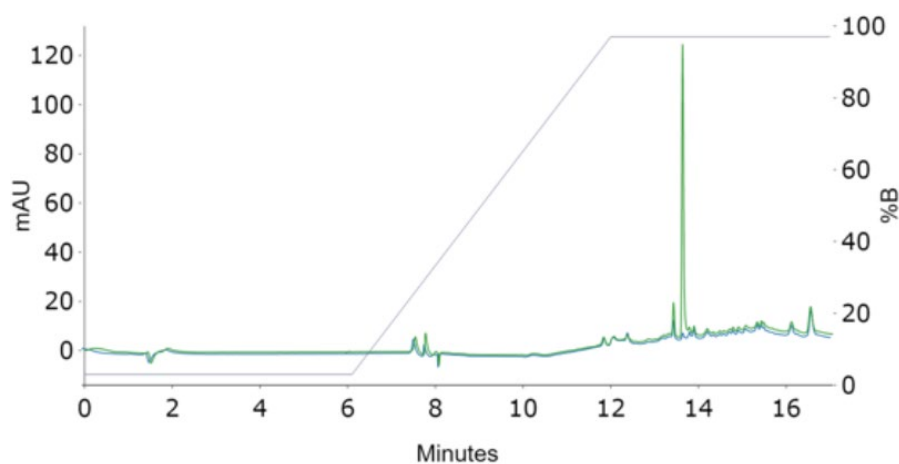
Retention time, min	Peak height, mAU	Peak area, mAU*s	Concentration based on peak height, µg/mL	Concentration based on peak area, µg/mL
6.66	10.8*	143*	44.4*	45.1*
6.65	11.6	155	47.7	48.8
6.66	11.7	157	47.9	49.6
6.64	11.7	156	47.9	49.2
Average	11.7	156	47.8	49.2
Std Dev	0.034	1.23	0.14	0.39
RSD, %	0.29	0.78	0.29	0.79

\*Excluded as an outlier based on Grubbs' test.

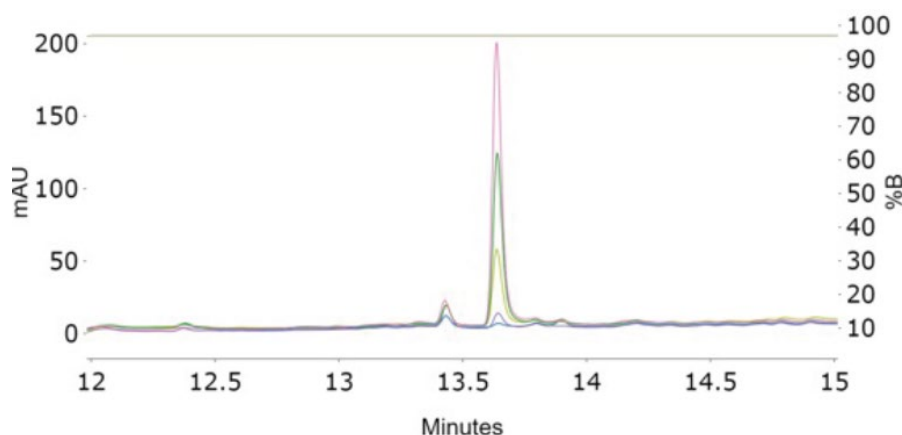
**Table 2.** Analysis of a Methanolic Extract of Wood Pre-treated with DCOIT.

## Quantitative Determination of ECONEA in Sea Water

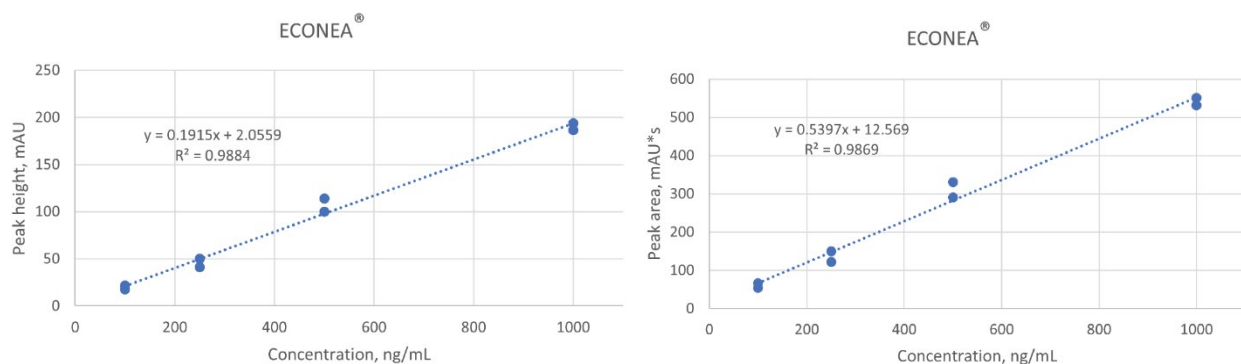
Initial experiments for the analysis of ECONEA were conducted using a 250 nL internal injection loop. To achieve better sensitivity, the assay was run with a larger external loop of 6  $\mu\text{L}$ . At a flow rate of 1  $\mu\text{L}/\text{min}$  and a sample injection volume of 6  $\mu\text{L}$ , the injection time was 6 min. Therefore, the solvent gradient program was initiated only after 6 min to allow complete injection of the sample into the column under isocratic conditions. The injection valve was then switched after 6 min to bypass the injection loop, so that the solvent mixture of changing composition could be delivered directly to the chromatographic column. Under these conditions, analyte concentrations of 100 ng/mL (286 nM) and 50 ng/mL could be quantified with signal-to-noise ratios above 10 and 6, respectively. Typical chromatograms at 50 ng/mL, 100 ng/mL, 250 ng/mL, and 500 ng/mL are shown in Figure 3 (A & B) and calibration curves are presented in Figure 4 (A & B).



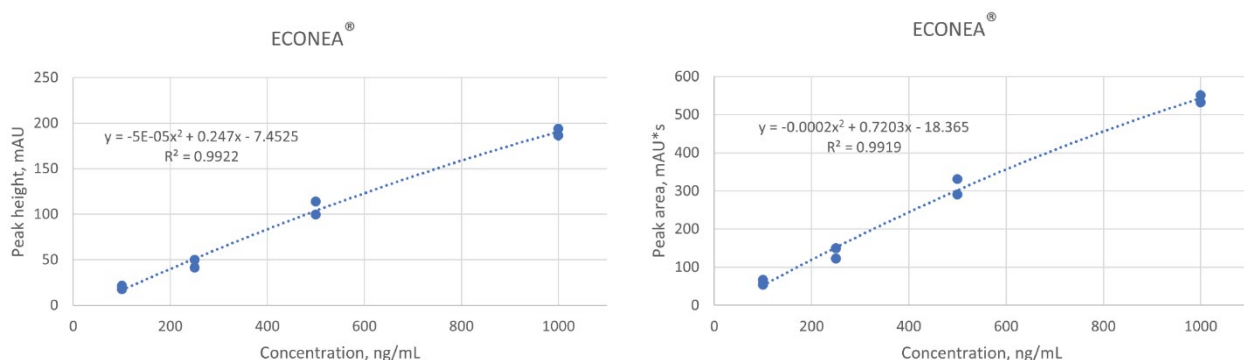
**Figure 3A.** Overlaid chromatograms of a blank sample (mobile phase A, blue trace) and ECONEA calibration standard in artificial sea water at 500 ng/mL (green trace).



**Figure 3B.** Overlaid chromatograms (in the time range of 12-15 min) of a blank sample (mobile phase A, blue trace) and ECONEA calibration standards in artificial sea water at 50 ng/mL (dark purple trace), 250 ng/mL (yellow trace), 500 ng/mL (green trace), and 1000 ng/mL (light purple trace).



**Figure 4A.** Determination of ECONEA in artificial sea water. The calibration curves were based on peak height (left) and peak area (right) with linear regression.



**Figure 4B.** Determination of ECONEA in artificial sea water. The calibration curves were based on peak height (left) and peak area (right) with quadratic regression.

Because of strong retention of the analyte on the chromatographic column and close match between the aqueous sample solution and the initial chromatographic mobile phase composition, the analyte was efficiently trapped and pre-concentrated on the column during the injection step. As a result, no substantial peak broadening was observed with the large injection loop. If desired, an even larger loop could probably be used (e.g., 10  $\mu$ L or 20  $\mu$ L). However, a larger sample loop would result in a longer total run time, as more time would be required for sample injection.

## Conclusions

The compact and portable Axcend Focus LC is an excellent solution for on-site quantitative analysis of DCOIT in wood extracts and ECONEA in ocean water in the concentration ranges of 20 - 80  $\mu$ g/mL and 100 - 1000 ng/mL, respectively.

The described methods can be used as a basis for further method development and for expanding, if necessary, the calibration ranges for quantitative analysis of these compounds.