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Determination of the Biocide Econea® in Artificial Seawater by Solid Phase Extraction and High Performance Liquid Chromatography Mass Spectrometry

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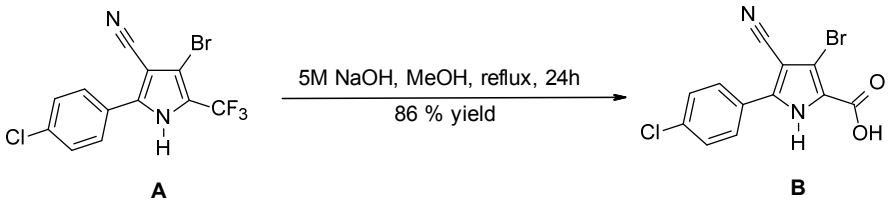
Abstract: Econea®, or 4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl)-1H-pyrrole-3-carbonitrile, is a new type of environmentally friendly anti-fouling compound used in the immersed coatings of commercial sea going vessels. This paper reports the development of a new analytical method to directly detect the active biocide Econea® in an artificial sea water matrix using liquid chromatography mass spectrometry. The developed method is both rapid and sensitive, with a limit of detection of 0.05 μg/L and a limit of quantitation of 0.17 μg/L in artificial seawater. The subsequent developed method was then applied to investigate the biocide's release from a commercially available Econea® containing paint immersed in artificial sea water over a 45-day period. It was found that the average release rate of Econea® from this paint was 4.3 ± 0.6 μg cm^{-2} d^{-1}.

Keywords: Econea®; 4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl)-1H-pyrrole-3-carbonitrile; paint; artificial seawater; LC-MS

1. Introduction

The anti-fouling of the immersed hull of commercial boats, ships and yachts is an important economic consideration for transporting products and people across the earth's maritime environment. Fouling is the build-up on the immersed hull of marine organisms such as barnacles, molluscs, slime and weed. Their adherence to the hull severely impacts its hydrodynamic efficiency with a consequent effect on performance and fuel efficiency [1]. To prevent this disadvantageous build-up, the immersed surface needs to be regularly treated with anti-fouling products. Historically, a range of anti-fouling products have been used that contain metal-based biocides (including copper, organotin and zinc), though these can lead to the build-up of elemental residues, and are increasingly the target of environmental legislation [2]. More recently, alternative technologies have been proposed that do not use metals, with one approach being biodegradable—and hence environmentally friendly—alternative biocides [3]. An example of this is Econea®, or 4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl)-1H-pyrrole-3-carbonitrile (see Scheme 1, compound **A**). Whilst the initial purpose of this biocide was to protect growing plants from infestation and attack by insects and plant mites [4], Econea® is also sold in anti-fouling paints by several different paint manufacturers [5]. Typically, paints are formulated using between 0.5 wt % and 9.9 wt % Econea® based on the total weight of the dry mass composition. When formulated at this level, excellent activity is observed against barnacles at 2 wt % [6]. Unlike metal-centred biocides, Econea® does not

accumulate in the marine environment, due to its rapid degradation in sea water (3 h and 15 h at 25 °C and 10 °C, respectively) [5] to 3-bromo-5-(4-chlorophenyl)-4-cyano-1H-pyrrole-3-carboxylic acid (BCCPCA) (see Scheme 1, compound **B**).



A **B**

Scheme 1. Hydroylsis of Econea® (or 4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl)-1H-pyrrole-3-carbonitrile) (**A**) to give 3-bromo-5-(4-chlorophenyl)-4-cyano-1H-pyrrole-3-carboxylic acid (BCCPCA) (**B**).

An ISO method for the indirect detection and quantification of Econea® in sea water samples has been reported [7]. The method is based on the use of high performance liquid chromatography with ultraviolet detection (HPLC-UV) at 280 nm to detect a hydrolysis breakdown product of Econea®, 3-bromo-5-(4-chlorophenyl)-4-cyano-1H-pyrrole-3-carboxylic acid (BCCPCA) (Scheme 1, compound **B**). As a seawater sample may contain a mixture of Econea®, as well as the breakdown product, in this method any Econea® in the sample is converted to BCCPCA, and the method quantitates this compound. The ISO method requires the samples to be degraded in a thermostatically controlled cabinet at 50 ± 5 °C for between 4 h and a maximum of 24 h. This method allows a level of quantification of BCCPCA of 1.87 µg/L.

As part of a research programme to develop novel marine coatings with enhanced controlled release characteristics, a sensitive method for detection of Econea® in sea water, in preference to BCCPCA, was sought. It has been shown previously that solid phase extraction followed by high performance liquid chromatography-electrospray ionisation-mass spectrometry (HPLC-ESI-MS) is a highly sensitive method for detecting and quantitating trace amounts of biocidal compounds in sea water [8]; work has also been reported using the more sensitive technique of LC-MS/MS [9]. This paper outlines a HPLC-ESI-MS method for the determination of Econea® in artificial seawater and its application to follow its release over time from a commercially available anti-fouling paint, and a range of polymer coatings bearing different dispersed amounts of Econea®. The commercially available paint is an anti-fouling paint that uses an ablative action, and contains two biocidal compounds, Econea®, and zinc pyrithione, as well as number of other components that aid its performance in other aspects of its utility [10].

2. Materials and Methods

2.1. Chemicals and Reagents

Analytical standard chemicals were purchased as follows: Econea® (PESTANAL®, Sigma-Aldrich, Gillingham, UK); acetonitrile and ammonium acetate (HPLC grade, Fisher Scientific, Loughbrough, UK); acetic acid (VWR, Lutterworth, UK); Tetra Marine SeaSalt (Amazon, Berkshire, UK). The sample of a commercial available Econea® containing paint was supplied by AkzoNobel, Gateshead, UK. Deionised water was prepared from distilled water purified using a Milli-Q-system (18 MΩ cm^{-1}) (Merck Millipore, Darmstadt, Germany).

2.2. Instrumentation

High performance liquid chromatography was performed on a Thermo Surveyor HPLC system (Thermo Fisher Scientific, Hemel Hempstead, UK) equipped with a degasser, quaternary pump and autosampler. Separation was achieved on a Phenomenex Luna C18 150 mm × 2.00 mm i.d. × 3 µm column with a 10 µL injection volume. The chromatography was performed using an isocratic

separation, 75% (A): 25% (B) at 200 μL min^{-1}. Elutant (A) was acetonitrile acidified with 1% (v/v) acetic acid and Elutant B consisted of filtered water with acetonitrile 5% (v/v) and 1% (v/v) acetic acid. The LC was coupled to an electrospray ion-trap mass spectrometer (MS) (Thermo LCQ Advantage) and was operated in negative ion mode. The ion source conditions were optimised using the automatic optimisation function of the MS software (LCQ Tune). This was done by introducing a 10 μg/mL solution of Econea®, in acetonitrile, by direct infusion into the mass spectrometer at a flow rate of 20 μL/min and tuning on the most abundant ion (M+2) at m/z 349. The electrospray ion source was operated at 280 °C. Other source conditions were sheath flow rate 30 (arbitrary units); ionisation spray voltage 4.5 kV; capillary voltage −4.00 V; tube lens offset 10.00 V; second octapole offset 11.00 V, first octapole offset 1.75 V and inter-octapole lens 16.00 V.

^{1}H-NMR spectra were obtained using a JEOL ECS 400 NMR spectrometer operating at a frequency of 400 MHz, using 8–32 scans, a relaxation delay of 5 s, and a flip angle of 45° (5 μs pulse). Spectra were Fourier transformed typically into 32,000 data points using standard exponential window with a line broadening factor of 0.2 Hz. ^{13}C spectra were obtained at a frequency of 100.53 MHz, from 128 to 1048 scans, a relaxation delay of 2 s, and a flip angle of 30° (2.7 μs pulse). Spectra were Fourier transformed typically into 64,000 data points using standard exponential window with a line-broadening factor of 0.5 Hz. All high resolution mass spectra (HRMS) were obtained from the EPSRC UK National Mass Spectrometry Service Centre, Swansea, UK.

2.3. Sample Preparation: Solid Phase Extraction

To investigate, and develop, a suitable solid phase extraction (SPE) protocol, a broad range of hydrophobic, hydrophilic, cation exchange and polymeric cartridges were compared. The highest recoveries were obtained with a C8 cartridge. Off-line SPE was performed by passing 10 mL of artificial sea water sample through a TELOS C8 (EC) (200 mg/3 mL) cartridge from Kinesis (St Neots, UK). The procedure was as follows. The SPE cartridge was washed with 6 mL acetonitrile, followed by 6 mL of filtered water containing 5% v/v acetonitrile. The cartridge was then conditioned with 6 mL acetonitrile followed by 12 mL of filtered water ensuring that the stationary phase did not run dry. Then, 10 mL of the aqueous sample containing Econea® was pipetted from the volumetric flask into a beaker. This sample was passed through the SPE cartridge, and the beaker was washed with approximately 3 × 3 mL of filtered water which was also passed through the SPE cartridge ensuring that the stationary phase did not run dry until the last aliquot. A vacuum was applied for at least 30 s to remove any residual water from the cartridge. The biocide was then eluted from the stationary phase using 8 mL of acetonitrile and quantitatively transferred to a 10 mL volumetric flask. Finally, 50 μL of internal standard of concentration 50 μg/L, which was dissolved in acetonitrile, was also added to the volumetric flask prior to making up to volume with acetonitrile. An aliquot of this solution was then transferred to a 2 mL autosampler vial prior to analysis.

2.4. Artificial Seawater Preparation

Artificial seawater was prepared from the sea water salt mix, 40 g of sea water salts were added to 1 L of distilled water, contents of the beaker were stirred until the sea water salts were dissolved. Any particulates were removed by vacuum through a Qualitative Filter Paper (Fisherbrand FILTER 1 QL100 240 mm). This provided artificial seawater (with the heavy metal component omitted) as per the appropriate ASTM [11].

2.5. Sampling under Controlled Release Conditions

A commercially available paint was supplied by AkzoNobel Ltd which is designed specifically for controlled release of Econea® into sea water. In addition, a solution (in butyl acetate and toluene) of a methacrylate polymer prepared from butyl methacrylate and isobornyl methacrylate using 2,2′-azobisisobutyronitrile as catalyst initiator was polymerised [12] with a starved feed methodology to disfavour polymer block formation [13]. From this polymer solution were created three samples of

dissolved polymer containing either 2, 15 or 38 percent composition by weight Econea® (as calculated from the mass of dried polymer product) by addition of powdered Econea® to the solution.

For all four polymer solutions, a 7 cm × 7 cm square coating was drawn down on to a glass panel using a 300 µm draw down bar. The panels were then dried for three days at room temperature in a low airflow extraction rack. The glass panel was then placed on an aluminium support in a beaker containing 250 mL of artificial sea water. The beaker was stirred using a stirrer hotplate calibrated to rotate at 240 rpm at room temperature. Each panel was prepared in triplicate. The volume of sample removed from the beaker is shown in Table 1.

Table 1. Average leach rates of Econea® from synthetic mixtures and a commercial coating.

Coating	Sampling Regime from Panels		Average Leach Rate/µg cm^{-2} d^{-1}
	Volume of Sample/mL	Volume of Eluant/mL	
2 wt % Econea®	100	10	0.4 ± 0.1
15 wt % Econea®	100	10	2.4 ± 0.2
38 wt % Econea®	10	10	7.9 ± 0.7
Commercial coating	10	10	4.3 ± 0.6

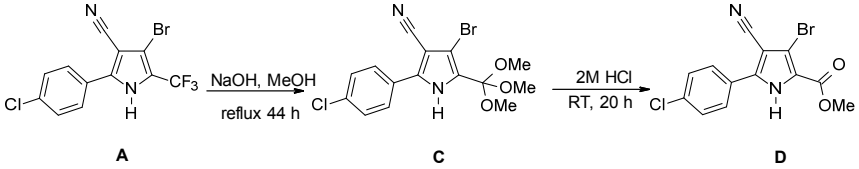
The leach rate was determined against a standard calibration graph using standards of Econea®, over the concentration range 0.5–100 µg/L, using 6 data points at concentrations of 0.5, 1, 5, 25, 50 and 100 µg/L. The limit of detection (LOD) and limit of quantitation (LOQ) was determined based on 3 or 10× standard deviation of the blank, respectively.

2.6. Synthesis of BCCPCA

To methanol (200 mL), Econea® (3.70 g, 1.1 mmol) and sodium hydroxide (5 M, 200 mL, 1000 mmol) were added. The mixture was refluxed for 24 h. Methanol was removed from the mixture using a rotary evaporator, and the solution diluted with water (200 mL). Hydrochloric acid (37%) was added until a solid precipitated as the pH of the solution reached pH 1. The solid was washed with water then dried to give BCCPCA (Scheme 1, compound **B**) (2.9533 g, 86% yield). m.p. 184–186 °C; ^{1}H-NMR (400 MHz; DMSO) δ 7.83 (d, *J* = 8.7 Hz, 2H, ArH) 7.63 (d, *J* = 8.7Hz, 2H, ArH). ^{13}C-NMR: (100 MHz, DMSO): δ 160.5 (C=O). The HRMS for $[M + H]^+$ was calculated as 324.9374, 325.9408, 326.9351, 327.9385, 328,9324; found 324.9383, 325.9413, 326.9356, 327.9387, 328.9325 (See Supplementary Information).

2.7. Synthesis of Internal Standard: Methyl Ester of BCCPCA

To 20 mL methanol, Econea® (3.57 g, 1.02 mmol) and powdered sodium hydroxide (0.83 g, 2.08 mmol) was added. The mixture was refluxed for 44 h. Sodium hydroxide (0.81 g, 2.03 mmol) and 5.51 g of methanol were then added to the reaction flask. After stirring for 3 days, hydrochloric acid (2 M; 20 mL, 40 mmol) was added until the pH was 1. The resulting solid was collected and washed with water to give an off white solid. Analysis by ^{1}H-NMR showed the solid to be a mixture of the trimethoxymethyl (see Scheme 2, compound **C**) and the methyl ester (see Scheme 2, compound **D**).



Scheme 2. Synthesis of internal standard, the methyl ester of BCCPCA (Compound D). Compound **A**, Econea® (or 4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl)-1H-pyrrole-3-carbonitrile); Compound **C**, tri(methoxy)methyl of BCCPCA (i.e., 3-bromo-5-(4-chlorophenyl)-4-cyano-1H-pyrrole-3-carboxylic acid); and Compound **D**, methyl ester of BCCPCA (i.e., 3-bromo-5-(4-chlorophenyl)-4-cyano-1H-pyrrole-3-carboxylic acid).

The crude white solid was added to a round bottom flask, to which hydrochloric acid (2 M; 80 mL, 160 mmol) was added, and then stirred overnight; the resulting solid was collected and washed with water. Analysis by ^{1}H-NMR showed the solid to have converted completely to the methyl ester (see Scheme 2, compound **D**). m.p. 165–167 °C; ^{1}H-NMR (400 MHz; DMSO) δ 7.83 (d, *J* = 8.7 Hz, 2H, ArH) 7.64 (d, *J* = 8.7Hz, 2H, ArH) 3.87 (s, 1H, OCH_3). ^{13}C-NMR: (100 MHz, DMSO): δ 159.5 (C=O), 52.6 (OCH_3); HRMS for $[M + H]^+$ calculated 338.9530, 339.9564, 340.9508, 341.9542, 342.9481; found 338.9533, 339.9563, 340.9506, 341.9537, 342.9476 (See Supplementary Information).

3. Results and Discussion

The analytical performance of the new method is shown in Table 2. The experimentally determined limit of quantitation (i.e., 0.17 µg/L) is of the order of 10× more sensitive than the ISO method (LOQ 1.87 µg/L) for the breakdown product of Econea®; this is not unexpected, given that this new method uses HPLC-MS. An example chromatogram of a sample containing 50 µg/L of Econea® and 50 µg/L internal standard is shown in Figure 1. Excellent baseline separation was observed for the internal standard and the Econea® peak.

Table 2. Analytical data for the determination of Econea®.

Compound	Calibration Range (µg/L)	Number of Data Points	Equation (y = mx + c)	Correlation coefficient, R^2	LOD (µg/L)	LOQ (µg/L)
Econea®	0.5–100	6	y = 0.0193x + 0.0269	0.9994	0.05	0.17

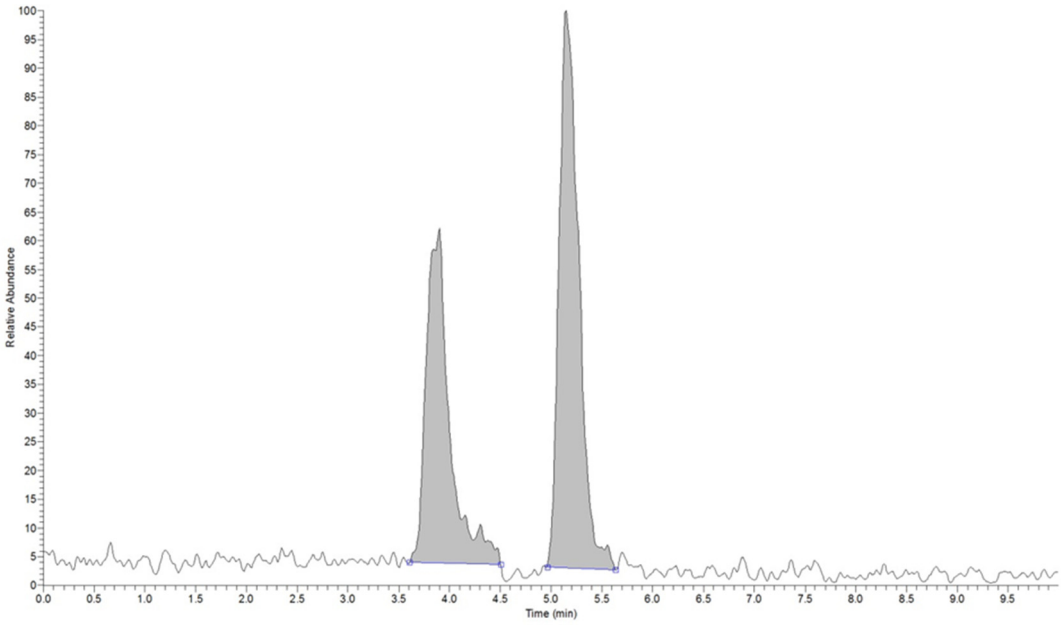


Figure 1. Example chromatogram of sample containing 50 µg/L internal standard (Tr = 3.95 min) and 50 µg/L Econea® (Tr = 5.25 min).

Subsequently, the leach rate of Econea® from the commercial paint was observed over a period of 45 days; samples were taken on days 1, 3, 7, 10, 14, 21, 24, 28, 31, 35, 38, 42 and 45 (as prescribed in ISO/CD15181-1) [14]. Based on the measured concentration of Econea® in the artificial seawater samples, the leach rate, R, (µg cm^{-2} d^{-1}) from the painted test plates was calculated:

$$R = \frac{C_{Econea} \times v \times 24}{t \times A} \quad (1)$$

where C_{Econea} is the concentration of Econea® released into the measuring container in µg/L; 24 is the number of hours per day; v is the volume in litres of artificial sea water (0.25 L); t is the time the test panel is immersed artificial sea water in hours (1 h); and, A is the surface area of the coating applied to the panel in cm^2 (49 cm^2). The results, displayed in Figure 2, show the variation over time; an average leach rate for the commercial paint of approximately 4.3 ± 0.6 µg cm^{-2} d^{-1} was observed for the duration of the study.

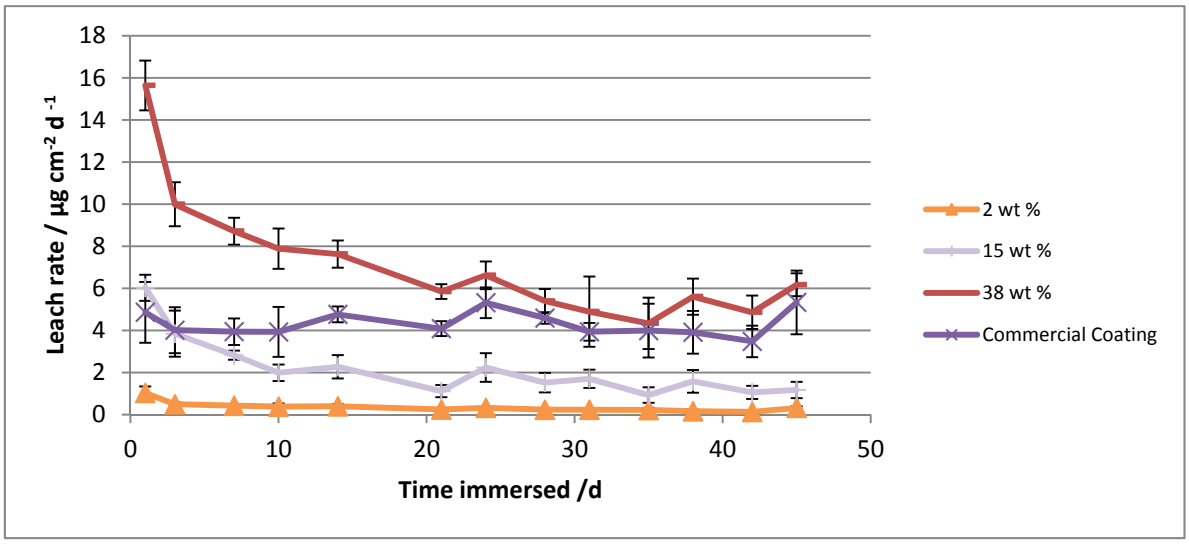


Figure 2. The leach rate of Econea®, from synthetic standards and a commercial coating, in artificial sea water. [The number of replicates per synthetic standard/commercial coating is 3. The error bars represent ± standard deviation].

4. Conclusions

The developed analytical method provides a reliable, robust and sensitive approach for the direct detection of Econea® from complex matrix samples despite its short half-life of 3 h in sea water. LC-MS combined with solid phase extraction is capable of operating with a detection limit of 0.05 µg/L in an artificial sea water sample. It should be noted that this method does not quantitate BCCPCA so whilst it enables the accurate determination of the level of Econea® in solution it cannot be used to make determinations of total leached biocide in the aqueous phase. Unlike the ISO method, which requires a dedicated rig that provides controlled release from coatings painted on rotating cylinders, this method used standard laboratory equipment. Additionally, this method is also applicable to a smaller coated area, and hence a smaller amount of biocide and host polymer need be prepared.

Supplementary Materials: The following are available online at http://www.mdpi.com/2297-8739/4/4/34/s1.

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Author Contributions: R.A.D., J.R.D. and J.J.P. conceived and designed the experiments with input from A.D.; R.A.D. performed the experiments; R.A.D., J.R.D. and J.J.P. analysed the data; A.D. contributed knowledge of the compound and paint; all authors contributed to the writing of the paper.

Conflicts of Interest: The authors (R.A.D., J.R.D. and J.J.P.) declare no conflict of interest. A.D. is employed by a company that produces paint containing the biocide.

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