Australian Government
Department of Defence
Defence Science and
Technology Organisation

Determination of Benzalkonium Chloride in Commercial Disinfectant Formulations by Quantitative NMR Spectroscopy

Nathan W. McGill

Human Protection and Performance Division
Defence Science and Technology Organisation

DSTO-TN-1132

ABSTRACT (U)

A biofouling treatment protocol for Royal Australian Navy vessels involves dosing with commercial disinfectant solutions containing the biocide benzalkonium chloride (BAC). In this work a single pulse proton Nuclear Magnetic Resonance (NMR) spectroscopic method was developed in order to permit the determination of BAC content in commercial formulations without suppression of the water resonance. The formulations were directly aliquoted into a NMR tube into which a stem coaxial insert, containing a reference solution of dimethylsulfone in deuterated lock solvent, was added. The samples were not filtered, diluted or concentrated and were quantified by comparison to a linear regression curve prepared from a commercially available standard. Eight samples from two commercial formulations were analysed with total BAC content determined to be 39-43 g/L for the Conquest® formulations and 40-60 g/L for the Quatsan® formulations.

RELEASE LIMITATION

Approved for public release

Published by

Human Protection and Performance Division
DSTO Defence Science and Technology Organisation
506 Lorimer St
Fishermans Bend, Victoria 3207 Australia

Telephone: (03) 9626 7000
Fax: (03) 9626 7999

AR-015-432
November, 2012

Conditions of Release and Disposal

Determination of Benzalkonium Chloride in Commercial Disinfectant Formulations by Quantitative NMR Spectroscopy

Executive Summary

A biofouling treatment protocol for Royal Australian Navy vessels involves dosing with commercial disinfectant solutions containing benzalkonium chloride (BAC); a molluscicide and antifouling chemical. In order to determine the efficacy of this treatment protocol, it is necessary to determine the concentration of BAC in each treatment solution. Conventional methods for BAC quantitation utilise liquid or gas chromatography. Whilst highly sensitive, these techniques require significant sample preparation such as filtration, solvent extraction, derivatisation and dilution. Nuclear Magnetic Resonance (NMR) spectroscopy is an alternative technique for the analysis of such samples, often without the requirement for solvent extraction, dilution or filtration. In this work, proton NMR spectroscopy was used to quantitate BAC content in commercial disinfectant formulations without suppression of the water resonance. The formulations were directly aliquoted into an NMR tube into which a stem coaxial insert, containing a reference solution of dimethylsulfone in D_2O, was added. The samples were not filtered, diluted or concentrated and were quantified by comparison to a linear regression curve prepared using a commercially available BAC standard. Eight samples from two commercial formulations were analysed with total BAC content determined to be 39-43 g/L for the Conquest® formulations and 40-60 g/L for the Quatsan® formulations. The methods and results presented herein will be used in a separate study to assess the efficacy of BACs as antifouling agents under realistic field conditions.

This page is intentionally blank

Contents

This page is intentionally blank

1. Introduction

An existing protocol for the in-water treatment of mussel biofouling within the internal sea water systems of RAN vessels is to dose with a disinfectant solution containing benzalkonium chloride (BAC); an anti-fouling compound and molluscicide. The specific anti-fouling activity of BAC is not yet well described. By analogy to other detergents, a likely mode of action is via the disruption of cellular membranes and the deactivation of enzymes critical to respiration and cellular metabolism. Previous DSTO laboratory trials examining the efficacy of commercial disinfectant solutions for treatment of mussel biofouling did not confirm the concentration of BAC in the treatment solutions.[1] In order to evaluate the efficacy of protocol, it is crucial to determine the concentration of BACs in the treatment solutions used. To achieve this, a method requiring minimal sample preparation is necessary to enable rapid determination of the batch-to-batch variation of BAC concentration. Quantitation of BACs is generally achieved by liquid chromatography with UV, fluorescence or mass spectrometric detection.[2-4] These methods require significant method development and sample preparation and are not suitable for the analysis of neat commercial formulations due to the high concentration of ingredients. Gas chromatography has been used to quantify BACs in environmental samples,[5] however, this approach does not enable short analysis turnaround times as extraction and chemical derivitisation is required. Additionally, analyses of BACs are often hampered by contamination issues as BACs are components of cleaning agents commonly used in analytical laboratories. NMR spectroscopy is an alternative technique for the analysis of samples such as commercial formulations. Modern NMR spectrometers exhibit large dynamic ranges, which permits the analysis of dilute and concentrated samples, thus reducing sample preparation requirements. As samples require minimal preparation and are fully contained during analysis, NMR spectroscopy facilitates rapid analysis turnaround times and reduces the likelihood of contamination from cleaning agents.

This report outlines the determination of total BAC content in eight samples, taken from two commercial disinfectant formulations, using quantitative ^{1}H NMR spectroscopy with external referencing. The method and results presented herein will be used in a separate study to further assess the efficacy of BACs as anti-fouling agents under realistic field conditions.

2. Results and Discussion

BAC is a mixture of alkylbenzyldimethylammonium chlorides of even-numbered alkyl chain lengths (Figure 1). Commercial mixtures of BAC are preponderantly composed of C12 and C14 homologues although C8, C10, C16 and C18 variants may also be present in low levels depending on the source.

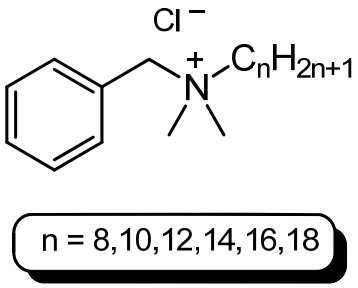

Figure 1. A generalised molecular structure of BAC

In order to quantify total BAC using linear regression, the samples and standards must have similar BAC profiles with respect to the ratio of the constituent homologues and the weighted average molecular weight. LC-MS was used to determine the BAC profile of the standard and samples by selectively monitoring the m/z of each homologue. The C8, 10 and 18 homologues were not considered during quantitation as they were found to constitute significantly less than 1% of the total BAC in the standard and the commercial formulations. The ratio of C12:C14:C16 was found to be 77:23:0 for the standard, 74:25:0 for Quatsan and 82:15:3 for Conquest, respectively (Appendix A1). These ratios were used to determine the weighted average molecular weight for the standard and samples. The standard was determined to have a weighted average molecular weight of 311 Da. The Conquest and Quatsan samples were found to have weighted average molecular weights of 310 and 311 Da, respectively. Given the similarity in the weighted average molecular weight for the standard and samples, it was suitable to quantify the samples using NMR spectroscopy and linear regression.

Total BAC content of the samples was calculated by integrating the NMR aromatic signal of BAC and comparing that value to a linear regression curve of BAC concentration versus total integral area for a series of standard solutions ranging 2.5-250 g/L total BAC (Appendix A2). The integral area of the BAC aromatic region was taken relative to an external reference of constant concentration as described in Section 2.1.3.

2.1 Experimental

2.1.1 Materials

A Sartorius ME5 analytical scale was used for weighing chemicals. Dimethyl sulfone (99.65%), benzalkonium chloride and acetonitrile were purchased from Sigma-Aldrich. D_2O (D 99.9%) was purchased from CIL. Formic acid was obtained from Fluka. All chemicals were used as received.

2.1.2 LC-MS Parameters

LC-MS data was acquired with a Hewlett-Packard Series 1100 MSD (+ESI) connected to an Agilent 1200 Series LC system comprising of an in-line degasser, binary pump, auto-injector, column heater and diode array detector. Data was collected with Agilent ChemStation LC/MSD software (Rev.B.03.01). Samples were eluted at 0.75 mL/min through a Supelco

Ascentis® Express 2.7 µm, 100 mm × 2.1 mm C18 HPLC column using gradient elution from 5% MeCN (+ 0.05% formic acid) to 90% MeCN (+ 0.05% formic acid) over 10 minutes.

2.1.3 NMR Spectroscopy Parameters

NMR spectra were acquired without suppression of the water resonance on a Bruker Avance Ultrashield 500 MHz spectrometer with a 5 mm BBI Z-GRD probe using Topspin software (V2.1). Table 1 outlines the parameters used for NMR data acquisition.

Table 1. Quantitative 1HNMR data acquisition parameters

Parameter	Value
Lock solvent	D_2O
Spin rotation	0 Hz
Measurement temperature	298 K
Pulse angle	90º
Preacquisition delay	6.5 us
Acquisition time	1.82 s
Relaxation delay	20 s
Number of scans	4
Sweep width	18 ppm
FID points	32k
Line broadening	0.3 Hz
Frequency of excitation	9 ppm

Raw FID files were multiplied by an exponential window function with a line-broadening factor of 0.3 Hz and then Fourier transformed to give frequency domain spectra. Spectra were referenced to the residual solvent signal and then the phase and baseline parameters were adjusted manually. Peak integration was extended symmetrically from the peak apex and terminated prior to reaching the ^{13}C satellites. The aromatic protons of BAC (7.3-7.5 ppm) and the methyl protons of DMS (3.1 ppm) were integrated. The integral values were exported to Microsoft Excel for calculations.

2.1.4 Stem Coaxial Inserts for NMR Spectroscopy

A stem coaxial insert was used to house the external reference solution.[6] The same NMR tube and insert was used for each measurement. The insert and NMR tube were thoroughly rinsed with distilled water and dried prior to analysis of the next sample. As the volume of the reference and analyte were identical for each sample measurement, the relative integral value of the analyte could be compared to a linear regression curve, which was acquired under identical conditions as described in Section 2.2.

2.2 NMR Spectrometric Analysis of Commercial BAC Formulations

Standards and samples were stored at 4 °C and allowed to warm to room temperature prior to analysis. Aqueous BAC standards (2.5, 5, 25 and 125 g/L) were prepared daily from a 250 g/L stock solution of BAC in distilled H_2O and analysed each day prior to sample analysis. A reference solution of DMS in D_2O was prepared at a concentration of 30 mg/mL. Samples were gently shaken for 15 minutes prior to subsampling. A 550 µL aliquot of sample was

directly added to an NMR tube and a stem coaxial insert, containing 50 µL of reference solution, was nested inside the NMR tube. Seven replicates of each sample were measured under the quantitative NMR conditions outlined in Section 2.1.3. The results are presented below in Table 2 with example spectra of the Conquest® and Quatsan® formulation presented in Figures 2 and 3.

Table 2. Concentration of BAC in commercial disinfectant formulations

type	sample	BAC (g/L)[1]
conquest	132	40.7 ± 0.9
	134	40.2 ± 1.2
	136	39.2 ± 1.8
	138	40.3 ± 2.4
	140	41.3 ± 1.3
	144	43.0 ± 2.1
quatsan	148	40.0 ± 1.6
	151	58.9 ± 3.7

[1] $p<0.05$, n=7

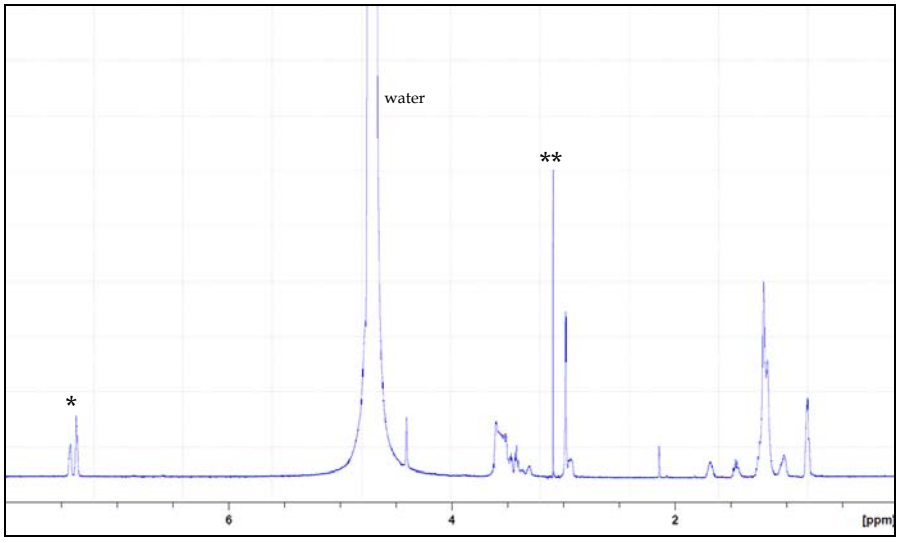

*Figure 2. Sample spectrum of neat Conquest formulation. *Aromatic BAC protons used for determination of total BAC content. **DMS external reference standard. The integral area of DMS is constant across all samples and was arbitrarily set to 1.000 (integrals not shown).*

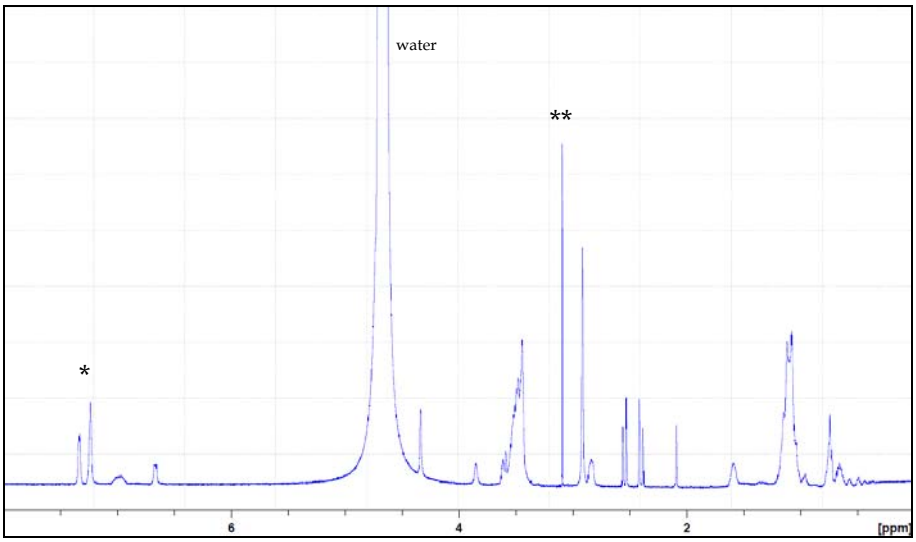

*Figure 3. Sample spectrum of neat Quatsan formulation. *Aromatic BAC protons used for determination of total BAC content. **DMS external reference standard. The integral area of DMS is constant across all samples and was arbitrarily set to 1.000 (integrals not shown).*

3. Summary

Single pulse ^1H NMR spectroscopy was used to quantify BAC content in commercial disinfectant formulations without suppression of the water resonance. The formulations were directly aliquoted into an NMR tube into which a stem coaxial insert, containing a reference solution of dimethylsulfone in D_2O, was added. The samples were not filtered, diluted or concentrated and were quantified by comparison to a linear regression curve prepared from a commercially available standard. Eight samples from two commercial formulations were analysed in duplicate. Total BAC content was determined to be 39-43 g/L for the Conquest® formulations and 40-60 g/L for the Quatsan® formulations. The methods and results presented herein will be used in a separate study to assess the efficacy of BACs as anti-fouling agents under realistic field conditions.

4. References

(1) Lewis, J.A.; Dimas, J. Treatment of biofouling in internal seawater systems - Phase 2. *DSTO-TR-2081*

(2) Kümmerer, K.; Eitel, A.; Braun, U.; Hubner, P.; Daschner, F.; Mascart, G.; Milandri, M.; Reinthaler, F.; Verhoef, J. *J. Chromatogr. A* **1997**, 774, 281-286.

(3) Prince, S. J.; McLaury, H.-J.; Allen, L. V.; McLaury, P. *J. Pharm. Biomed. Anal.* **1999**, 19, 877-882.

(4) Radke, M.; Behrends, T.; Förster, J.; Herrmann, R. *Anal. Chem.* **1999**, 71, 5362-5366.

(5) Suzuki, S.; Nakamura, Y.; Kaneko, M.; Mori, K.; Watanabe, Y. *J. Chromatogr. A* **1989**, 463, 188-191.

(6) McGill, N. W.; Thomson, S. A. *DSTO-TR-2748 September 2012*.

Appendix A: Supplementary Information

A1. LC-MS Chromatograms

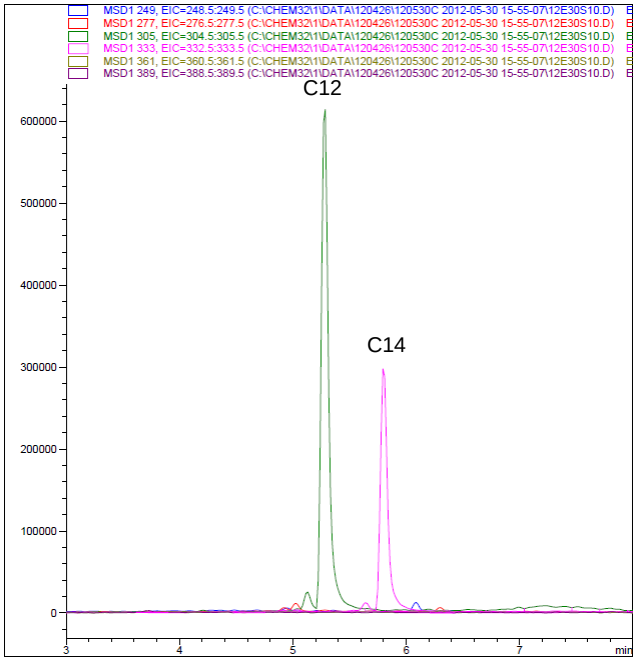

Figure A1.1. Extracted ion chromatogram of the BAC standard (1 ppm in water) showing the relative ratio of the C12 and C14 homologues

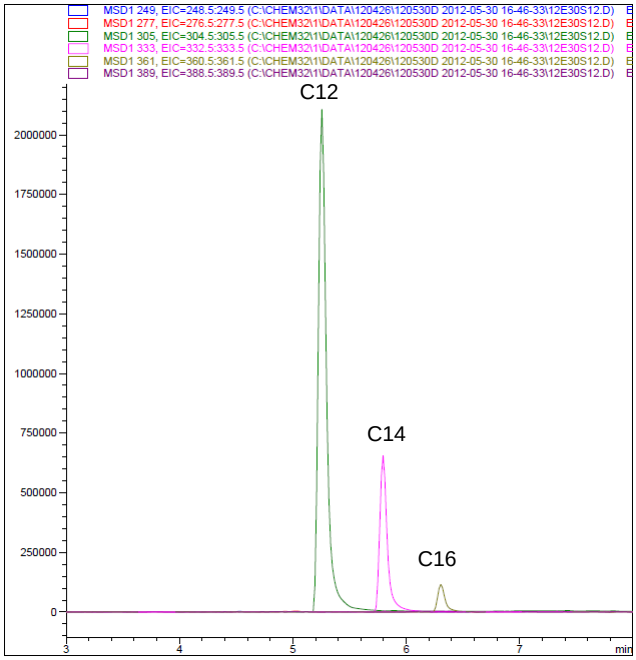

Figure A1.2. Extracted ion chromatogram of the Conquest formulation (1 ppm in water) showing the relative ratio of the C12, C14 and C16 homologues

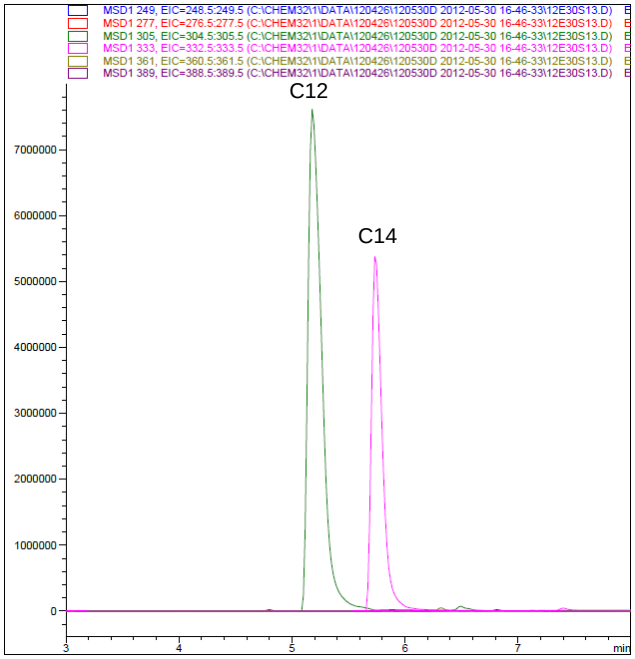

Figure A1.3. Extracted ion chromatogram of the Quatsan formulation (0.1 ppm in water) showing the relative ratio of the C12 and C14 homologues

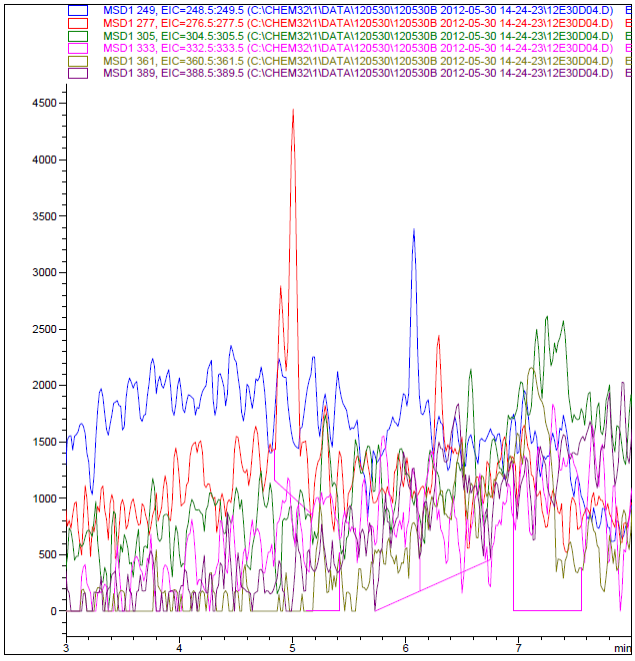

Figure A1.4. Extracted ion chromatogram of distilled water (blank) showing the absence of BAC

A2. Linear Regression Curves

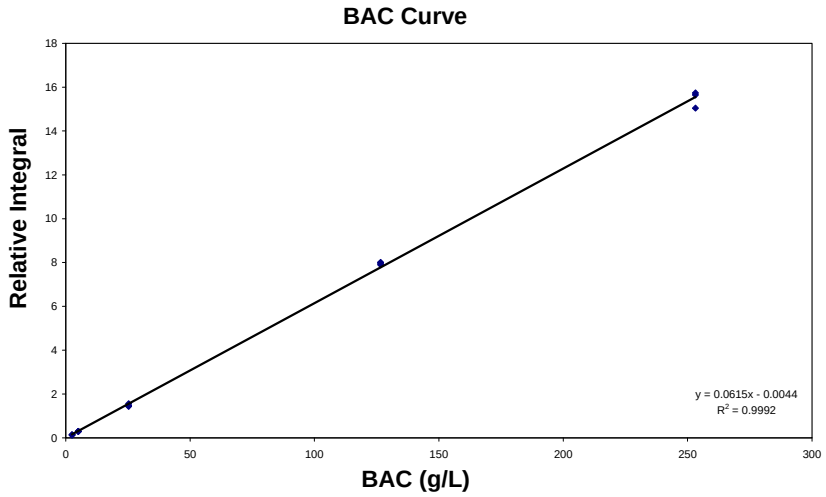

Figure A2.1. Linear regression curve 1 used for quantitation of samples 132-139; 148-150

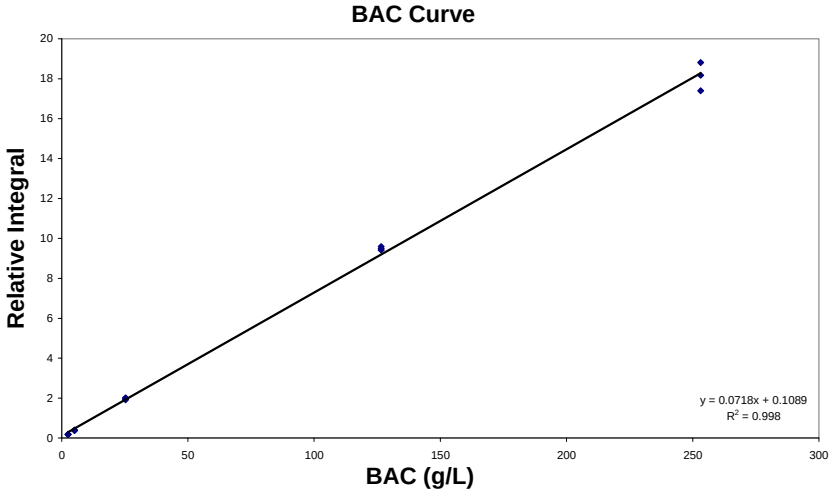

Figure A2.2. Linear regression curve 2 used for quantitation of samples 140-145; 151

DEFENCE SCIENCE AND TECHNOLOGY ORGANISATION
DOCUMENT CONTROL DATA

1. PRIVACY MARKING/CAVEAT (OF DOCUMENT)

2. TITLE

Determination of Benzalkonium Chloride in Commercial Disinfectant Formulations by Quantitative NMR Spectroscopy

3. SECURITY CLASSIFICATION (FOR UNCLASSIFIED REPORTS THAT ARE LIMITED RELEASE USE (L) NEXT TO DOCUMENT CLASSIFICATION)

Document	(U)
Title	(U)
Abstract	(U)

4. AUTHOR(S)

Nathan W. McGill

5. CORPORATE AUTHOR

DSTO Defence Science and Technology Organisation
506 Lorimer St
Fishermans Bend Victoria 3207 Australia

6a. DSTO NUMBER	6b. AR NUMBER	6c. TYPE OF REPORT	7. DOCUMENT DATE
DSTO-TN-1132	AR-015-432	Technical Note	November 2012

8. FILE NUMBER	9. TASK NUMBER	10. TASK SPONSOR	11. NO. OF PAGES	12. NO. OF REFERENCES
2012/1109743/1	-	-	9	6

13. DSTO Publications Repository

http://dspace.dsto.defence.gov.au/

14. RELEASE AUTHORITY

Chief, Human Protection and Performance Division

15. SECONDARY RELEASE STATEMENT OF THIS DOCUMENT

Approved for Public Release

OVERSEAS ENQUIRIES OUTSIDE STATED LIMITATIONS SHOULD BE REFERRED THROUGH DOCUMENT EXCHANGE, PO BOX 1500, EDINBURGH, SA 5111

16. DELIBERATE ANNOUNCEMENT

No Limitations

17. CITATION IN OTHER DOCUMENTS Yes

18. DSTO RESEARCH LIBRARY THESAURUS

NMR spectroscopy; biofouling; benzalkonium chloride

19. ABSTRACT

A biofouling treatment protocol for Royal Australian Navy vessels involves dosing with commercial disinfectant solutions containing the biocide benzalkonium chloride (BAC). In this work a single pulse proton Nuclear Magnetic Resonance (NMR) spectroscopic method was developed in order to permit the determination of BAC content in commercial formulations without suppression of the water resonance. The formulations were directly aliquoted into a NMR tube into which a stem coaxial insert, containing a reference solution of dimethylsulfone in deuterated lock solvent, was added. The samples were not filtered, diluted or concentrated and were quantified by comparison to a linear regression curve prepared from a commercially available standard. Eight samples from two commercial formulations were analysed with total BAC content determined to be 39-43 g/L for the Conquest® formulations and 40-60 g/L for the Quatsan® formulations.