Measurement of O-phthalaldehyde (OPA) by High Pressure Liquid Chromatography (HPLC) with Post Column Derivatization

Al E. Watkins*, Danelle Dees* and Tabatha Butler*

*A2Research, John C. Stennis Space Center, Stennis, MS, USA, al.e.watkins@nasa.gov, danelle.c.dees@nasa.gov, tabatha.butler-1@nasa.gov

ABSTRACT

O-phthalaldehyde (OPA) is commonly used as a high-level disinfectant in the medical industry. On the International Space Station (ISS), waste heat loads are removed by an Internal Active Thermal Control System (IATCS) which is a water based system in conjunction with the an EATCS (External ATCS), ammonia based system. OPA is used in the IATCS water based system as a biocide to prevent negative impacts to coolant flow, heat transfer, and corrosion. Therefore the concentration of the OPA in the coolant fluid is critical to maintain optimum function of the IATCS [1]. The previously used method for validating OPA concentration had been proprietary and is no longer available to NASA. To address the critical need for ISS, a simple and inexpensive process was developed to determine OPA concentration in water by reversing an established method used to determine glyphosate concentrations in water by HPLC using post column derivatization.

Keywords: O-phthalaldehyde, HPLC, NASA, derivatization, ISS

1 BACKGROUND

On the International Space Station (ISS), systems produce waste heat which needs to be transferred from the ISS to space to achieve thermal control and maintain components at acceptable temperatures. The Internal Active Thermal Control System (IATCS) is a critical system used to facilitate heat transfer aboard the (ISS). The IATCS aboard the ISS is primarily responsible for the removal of heat loads from payload and system racks. This also includes, heat loads from crew members, experiments, electrical equipment and other systems generate heat inside the modules of ISS that also has to be rejected. To accomplish this, the IATCS consists of loops that circulate water through the interior to collect the excess heat. The IATCS is a water based system which works in conjunction with the EATCS (External ATCS), an ammonia based system, to facilitate heat transfer aboard ISS. The purpose of the Thermal Control System of ISS is to keep all internal and external equipment and payloads at temperatures within their specific thermal requirements. The IATCS circulates water through two independent loops inside the modules, a Low Temperature Loop and a Moderate Temperature Loop in order to segregate the heat loads, to simplify heat load management and to add redundancy in case of equipment failures. Water is used because it is an efficient heat transfer fluid and presents no danger to crew members in the event of an internal leak.

Since 2001, growth and proliferation of microorganisms in the IATCS on ISS has been of significant concern. NASA and Boeing condcuted testing and assessments of a variety of biocides, to determine bacterial disinfection capability, material compatibility, stability (rate of oxidative degradation and identification of degradation products), solubility, application methodology, impact on coolant toxicity hazard level, and impact on environmental control and life support systems to identify a prioritized list of acceptable biocidal agents. O-phthalaldehyde, OPA, an aromatic dialdehyde compound with the formula $C_6H_4(CHO)_2$ was selected as the optimum antimicrobial. OPA is commonly used as a high-level disinfectant (for example, for sterilization of heat sensitive medical instruments) that demonstrates effective microbicidal activity against a range of microorganisms including mycobacteria, gram-negative bacteria and spores.

Upon selection for use, OPA was thoroughly evaluated and proven effective for use in the water based portion of the coolant system as the preffered microbial growth inhibitor for use in the ISS IATCS, replacing silver as the choice antimicrobial, and was approved for use at concentrations $<$109 ppm.

The concentration of the OPA in the coolant fluid is critical to the process and previously used methods for developing, analyzing and validating the OPA concentration. The previously used method for developing, analyzing and validating the OPA concentration had been proprietary process which no longer was available to NASA. A subsequent literature search could only provide a technical method that was both labor intensive and involved using hazardous materials (hydrazine dissolved in concentrated sulfuric acid).

Therefore, NASA Environmental Laboratory located at the John C. Stennis Space Center (SSC), worked closely with NASA Kennedy Space Center (KSC) to develop a simple, inexpensive, and reliable methodology for analyzing low- to mid- level concentrations of OPA for use in IATCS coolant water.

2 INTRODUCTION

Accumulation of microorganisms on surfaces inside IATCS could result in material degradation. Should the IATCS become damaged, crew health and safety are at risk. OPA is a disinfectant that effectively inhibits the growth and recovery of viable microorganism. An OPA water solution is required and used as a high level disinfectant to effectively inhibit the growth and recovery of viable microorganisms in the IATCS coolant to prevent microbial negative impacts to coolant flow, heat transfer, and corrosion. The concentration of the OPA in the coolant fluid is critical for maintaining proper functionality of IATCS. To address this acute need for ISS, an analytical process was developed which could verify a known concentration of the disinfectant OPA.

A National Institute of Standards and Technology (NIST) traceable EPA method 547 for quantitating glyphosate concentration in water, which uses High Pressure Liquid Chromatography (HPLC) method that uses OPA to convert glycine (not glyphosate) in a post-column reactor into a compound that can be more readily detected, was reversed engineered. By reversing the premise of this process, glycine is reacted in excess with the OPA solution along with 2-mercapto-ethanol. This developed and validated method to analyze OPA offers a new effective, low cost HPLC method that substantially reduces the future process waste stream and avoids potential health hazard effects from previously identified processes.

This simple inexpensive, NIST traceable process to analyze OPA that does not require highly hazardous chemicals nor does it have a hazardous waste stream that is costly to discard.

3 TECHNOLOGY

NASA SSC operates a full scale Environmental laboratory, and utilizes Environmental Protection Agency (EPA) methodologies for most analytical processes. EPA Method 547, entitled *Determination Of Glyphosate In Drinking Water By Direct-Aqueous injection Hplc, Post-Column Derivatization, And Fluorescence Detection,* is a widely accepted method. Method 547 effectively identifies and determines Glyphosate (N-phosphonomethyl glycine) concentrations in drinking water matrices. Once Glyphosate is eluted from the column, it is oxidized using hypochorite solution to form glycine. OPA, used in conjunction with either N,N-dimethyl-2-mercaptoethylamine hydrochloride or thioflur, derivitize the glycine to form a highly fluorescnet isoindole compound [1] [2].

NASA SSC's Environmental Laboratory calibrated, using known concentrations of OPA solutions, derivitized and performed post column reaction of the OPA solutions with glycine and 2-mercapto-ethanol. The resultant reaction complex was used to determine the OPA concentration with accuracy and precision. Calibrations were established, proficiency studies (IDOPs) and a method detection limit (MDL) study were analyzed to demonstrate the precision and accuracy. This developed and validated method to analyze OPA offers a new effective, low cost HPLC method that substantially reduces process waste. Additionally, the final product of the reaction is environmentally friendly. Glycine is an active chemical neutralizer used in the medical industry for disposal of expired, unused high-level disinfectants, such as OPA. Because Glycine, a primary amino acid which is a building block for protein, OPA readily reacts with it and "attacks" the protein, and quickly becomes suspended/neutralized [3] [4].

4 DEMONSTRATION OF PROFICIENCY

4.1 Calibration and Linearity

The calibration range for this method was established specifically for the optimum concentration of OPA in IATCS solution. A five point calibration range was used with the low standard concentration set at 100 mg/L, and a high standard of 500 mg/L. The coorelation coefficient for the calibration was determined to be 0.99979. Second source calibration verification standards were used to verify the calibration. A 300 mg/L second source standard was analyzed and reported at 293.3 mg/L, a 97.8% recovery.

4.2 Precision and Accuracy Qualification

Upon establishing and verifiying calibration procedures for OPA concentration determination, precision and accuracy studies were performed over three non-consecutive days, using a 250 mg/L standard, four replicates were analyzed per sample run for a total of 12 data points. The average recovery for this study was reported to be was analyzed four times each . Over the course of three separate runs, the average result reported for the 250 mg/l standard was 255 mg/L, or 102% recovery. The relative standard deviation was 8.02 for the three data sets.

4.3 Establishment of Method Detection Limit

Method detection limit (MDL) studies were performed over the course of three months utilizing the lowest standard on the calibration curve. A 100 mg/L standard was analyzed in three separate analytical runs to establish an MDL of 7.4 mg/L. MDLs were established following guidelines set for in the *US Code of Federal Regulations* Title 40 Chapter 1 [5].

5 CONCLUSIONS

OPA has demonstrated effective inhibitory growth and recovery of viable microorganism in the IATCS coolant when the concnetration of OPA is greater than the minimum inhibitory concentration of 100 ppm. Levels of up to 300 mg-OPA/L are also used in various loops of the IATCS aboard the ISS to keep microbiological populations in check.. Standard validation practices for OPA concentration have to be accessible to ensure effectiveness of implmentation.

The implementaion of OPA in the IATCS has culminated in a safe, compatible, effective, and sustinable method to control microbioal growth in the ISS IATCS to prevent microbial impacts to the coolant flow, heat transfer, corrosion and crew health and safety. This method enables a cost-effect apporach for OPA qualification, which could potentially used future space-based and medical application.

REFERENCES

[1] American Institute of Aeronautics and Astronautics, International Space Station (ISS) Internal Active Thermal Control System (IATCS) New Biocid Selection, Qualification, and Implemetation. Mark E. Wilson, Boeing, Harold E. Cole, Boeing, Tony Rector, John Steele, and Jerry Varsik, Hamilton Sundstrand Space Systems International, Inc., Windsor Locks, CT

[2] METHOD 547-“Determination of Glyphosate in Drinking Water by Direct-aqueousinjection HPLC, Post-Column Derivatization, and Fluorescence Detection” (applicable to glyphosate), which is incoporated by reference in accorance with 5 U.S.C. 552(2) and 1 CFR part 51, July 1990 T.W. Winfield W.J. Bashe (Technology Applications Inc.) T.V. Baker (Technology Applications Inc.), Environmental Monitoring Systems Laboratory Office of Research and Development, US Environmental Protection Agency, Cincinnati, OH

[3] Rutala, W.A. and D.J. Weber, “New Disinfection and Steriization Methods”, Emerging Infectious Diseases, Vol 7, No. 2, 348-353, 2001.

[4] Rutala, W. A., and D. J. Weber, D. J. “Sterilization, high-level disinfection, and environmental cleaning”. *Infectious disease clinics of North America,* 25(1), 45-76, 2011.

[5] *U.S. Code of Federal Regulations*, Title 40: Protection of the Environment, Volume 22, Chapter 1, Subpart D-Reporting and Record Keeping, Part 126, Appendix B (7-1-09 Edition) .