CALIFORNIA STATE UNIVERSITY, NORTHRIDGE

DEVELOPMENT OF THREE FOOD ANALYSIS AND CHEMISTRY EXPERIMENTS USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY AND ATOMIC ABSORPTION SPECTROPHOTOMETRY FOR CSUN FOOD SCIENCE STUDENTS

A graduate project submitter in partial fulfillment of the requirements for the degree of

Master of Science in Family and Consumer Sciences

by

Daria Maria Baciu

May 2015

The graduate project of Daria Maria Baciu is approved:

Julie Ellis, M.S.	Date
Elizabeth Sussman, Ph.D., R.D.	Date
Terri Lisagor, Ed.D, MS, RD, Chair	Date

California State University, Northridge

DEDICATION

This graduate project is dedicated to:

My family and loved ones who constantly supported me throughout the process of developing this project. Thank you for believing in me and for reminding me that everything is possible.

Dr. Terri Lisagor, for constant guidance and immense help with the completion of this project, and Dr. Claudia Fajardo-Lira, for giving me the opportunity to take on this project.

ACKNOWLEDGMENT

I would like to thank my committee members who supported my efforts in writing this graduate project.

To my chair, Terri Lisagor, Ed.D., M.S., R.D.,

To Julie Ellis, MS.,

To Elizabeth Sussman, Ph.D., R.D.,

Thank you for your valuable guidance and support, I could not have done it without you.

I would also like to thank Diem Nguyen, for working along with me in troubleshooting the HPLC and AAS instruments.

TABLE OF CONTENTS

Signature Page ii
Dedication iii
Acknowledgement iv
Abstract v
CHAPTER I – INRODUCTON 1
Statement of the Problem 1
Purpose 1
Definitions 2
Assumptions 2
Limitations 3
CHAPTER II – REVIEW OF LITERATURE 4
History of Food Industry and Food Science Education in the United States 4
Food Quality and Safety Using Analytical Tools 7
HPLC Background and Use in the Food Science Industry 9
AAS Background and Use in the Food Science Industry 12
CHAPTER III – METHODOLOGY 15
CHAPTER IV – RESULTS 18
CHAPTER V – DISCUSSION 19
Discussion of Findings and Modifications 19
Implications 20
Conclusion 21
REFERENCES 22
APPENDIX A 26
APPENDIX B 42
APPENDIX C 51
APPENDIX D 58

ABSTRACT

DEVELOPMENT OF THREE FOOD ANALYSIS AND CHEMISTRY EXPERIMENTS USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY AND ATOMIC ABSORPTION SPECTROPHOTOMETRY FOR CSUN FOOD SCIENCE STUDENTS

by

Daria Maria Baciu

Master of Science in Family and Consumer Sciences

The purpose of this project was to develop three Food Analysis and Chemistry experiments for Food Science students at California State University, Northridge (CSUN), using available analytical instruments such as High Performance Liquid Chromatography (HPLC) and Atomic Absorption Spectrophotometry (AAS). The HPLC experiments include determination of capsaicinoids in peppers, and determination of coumarin in ground cinnamon. The AAS instrument was used to determine the amount of lead in vinegar. The proposed experiments will provide CSUN Food Science students with necessary analytical laboratory skills to succeed in their future careers.

CHAPTER I

INTRODUCTION

High Performance Liquid Chromatography (HPLC) and Atomic Absorption Spectrophotometry (AAS) are two important tools of analytical chemistry that are widely used in the chemical, pharmaceutical, and food industry. Chemical analysis of food plays a critical role in the quality assurance program, as well as in formulating and developing new products (Nielsen, 2010). The safety and quality of food products is of utmost importance for both regulatory agencies and consumers. Therefore, evaluation and monitoring of foods at the molecular level is essential for the food industry so it can satisfy governmental and consumer demands. HPLC and AAS have many applications in food chemistry and have been used to analyze food components such as carbohydrates, amino acids, fats, vitamins, pigments, food additives, allergens, pesticides, and other organic compounds. Being familiar with these powerful analytical tools would benefit those who want to have a successful career in the food science field.

Statement of the Problem

The Food Science Department at California State University, Northridge (CSUN) has limited Food Science classes to offer. The lack of up-to-date Food Chemistry and Analysis experiments is one of the contributing factors to this problem. Performing food analysis using modern analytical techniques will prepare the students for the competitive job market.

Purpose

The purpose of this project is to create three Food Chemistry and Analysis experiments, which incorporate the use of analytical instruments such as HPLC and AAS.

Food Science professors can use the proposed experiments in Food Chemistry classes in order to teach the principles, functionality, and operation methods of important analytical instruments such as HPLC and AAS.

Definitions

- AAS: Atomic Absorption Spectrophotometer uses very high temperatures to decompose any sample into atoms. This will be measured by their characteristic wavelength.
- Analyte: a substance or chemical compound that is undergoing quantitative chemical analysis
- HPLC: High Performance Liquid Chromatography is an analytical instrument that uses high pressure to force solvent through a column with very fine particles; this will give a high-resolution separation of the sample.

Assumptions

This project, which includes the development of three Food Chemistry and Analysis experiments, was based upon the following assumptions:

- Food Science students who will perform the experiments have all the prerequisites fulfilled (Chemistry, Biology, Introductory Food Science, Introductory Food Chemistry).
- The laboratory instructor has knowledge about the instruments used (HPLC, AAS) and is able to provide students with an overview on how to operate the machine.
- The experiments are based on previously developed methods. However, necessary changes to accommodate CSUN's Food Chemistry laboratory will be made.

- All experiments use scientific language and are usable for the students.
- Students understand the experiments.
- The reagents are of required quality and purity.
- Analyses were performed correctly.

Limitations

This project will contribute to the creation or improvement of a graduate level Food Analysis class. However, certain limitations exist:

- The HPLC and AAS instruments need to be run periodically to ensure proper functioning, and qualified personnel are needed.
- These experiments need to be conducted individually so that every student understands and gets to practice using each instrument, however it will take more than one class period to do so.
- The reagents used in the experiments need to have a specific purity, which makes them more expensive.
- As new analytical techniques and methods of analysis are developed, these experiments need to be reviewed and updated periodically.

CHAPTER II

REVIEW OF LITERATURE

History of Food Industry and Food Science Education in the United States

The food science industry has provided Americans with the most plentiful, least expensive, and safest food supply in the world from the early 1900's to the present (Arnold et al., 2000). During the first 30 years of the 20th century, the food industry was concerned with getting enough food to the people by developing methods of preserving food, and trying to prevent adulterations. During the Great Depression and World War II, consumers wanted more meat, dairy products, and sweets; however, certain food ingredients were scarce. After 1945, more women started working and convenience was very important. It was during this time when food scientists were in higher demand than ever before. Between 1965 and 1980, there was an era of transition, when consumers started to be more involved in the food industry, product standards changed, food quality and content was overseen more tightly by the FDA and USDA, and food labeling was contributing to more nutritional knowledge for the consumers. After 1980, the food industry became more international, and seasonal foods started to become more available all year round. The consumers started to become more concerned with the safety of their food, and new testing methods emerged to assist the industry and regulatory agencies in proving safe and fresh foods (Arnold et al., 2000).

As the demand for nutritious, fresh, and safe foods increased, the Food Science programs offered by universities in the United States expanded. Even though there were a few universities offering Food Technology programs from the 1920s to 1950s, the number of Food Science programs increased significantly in the 1950s and 1960s

(Iwaoka, 2011). The Institute of Food Technologists (IFT) published a "model curriculum" in 1958. However, the first set of educational standards for Food Science programs was not developed until 1966 (Hartel, 2002). This set of standards served as mere guidelines rather than specific curriculum and course content requirements. In 1977, the Institute of Food Technologists (IFT) formalized the IFT Minimum Standards, and Food Science programs needed the IFT approval in order for their students to qualify for IFT Scholarships (Hartel, 2006). The 1977 IFT Minimum Standards required specific courses in various aspects of Food Science such as Microbiology, Chemistry, and Engineering, as well as the fulfillment of core background courses in Chemistry, Biology, Physics, and Mathematics. In 1992, the IFT reviewed the Minimum Standards and added communications, critical thinking, statistics, and computer literacy as necessary educational skills (Iwaoka, 2011). In 1997, the IFT formed a Task Force charged to "review and recommend outcome-based guidelines as compared to minimum standards to inspire excellence in food science education" (Hartel, 2002, p. 3). After several years, in 2001, the IFT Task Force developed new guidelines entitled The 2001 IFT Education Standards. An IFT Committee on Higher Education (CoHE) was appointed to implement the IFT Education Standards and to review all Food Science programs every 5 years (Hartel, 2006). These new guidelines included new course content requirements to include Food Law, Quality Control, and Sensory Analysis. The programs have to document that they cover all the required content stipulated by the IFT Education Standards, but now they have the flexibility to be creative in how they cover the material. According to the IFT Education Standards, there is no need for specific courses as long as the program documents that all the required competencies are being met, even if it is

from classes that belong to different departments of the university. Besides specific learning outcomes, the program must have an assessment program that evaluates whether the learning outcomes have been met. Also, the IFT-approved Food Science programs need to implement a self-evaluation and self-improvement program. In 2006, during the five-year review and re-approval process of the 2001 IFT Education Standards, the members of CoHE noticed that some approved programs did not assess their programs and no improvements were made (Iwaoka, 2011). A new committee, the Higher Education Review Board (HERB) [formerly known as CoHE], formed a Task Force to review the effectiveness of the 2001 Educational Standards and to consider possible revisions. The heads and chairs of Food Science programs were able to make comments and vote upon the recommendations made by the Task Force. Out of this, a major change from the 2001 Guidelines was the requirement for each approved program to submit an annual report in order to help HERB conduct an assessment in a timely fashion.

The changing nature of education provides that students are able to use the information learned in a specific situation, such a laboratory experiment, to solve problems in different real life situations; to solve problems that have not yet appeared using information they already know; and to synthesize new solutions using pieces of information from multiple sources. It is also important for students to learn to communicate effectively in different ways with a variety of populations. The newly revised guidelines are meant to help the IFT-approved Food Science programs to move in this direction of educating the future food scientists.

A food company that wants to excel in the emerging market has to possess highly skilled personnel who bring to the workforce educational and technical skills, as well as

resourcefulness, creativity, and the ability to interact and communicate effectively with a diverse clientele (Chikthimmah & Floros, 2007). The United States was projected to experience a shortage in qualified food scientists. It was estimated that there were 52,000 annual job openings between 2005 and 2010; however, there were still 2,700 unfilled positions every year during that period (Chikthimmah & Floros, 2007). Adding to the shortage problem, the United States is experiencing declining enrollment in graduate Food Science programs (Roberts, Robbins, McLandsborough, & Wiedmann, 2010). This trend is most probably due to the fact that well-qualified undergraduates are offered competitive summer internships that lead to lucrative jobs in the industry, diminishing the need to pursue an advanced Food Science degree. In addition, there is a need for food scientists with interdisciplinary training such as veterinary medicine, bioinformatics, chemistry, and nutrition. Weller, Robbins, Elmore, and Weidmann (2015) mention that it is critical to recruit and retain qualified students to graduate-level programs in order to satisfy the industry, academia, and government needs. As a result, Cornell University offers a Master of Professional Studies Program, which is a course-based graduate degree as an alternative to research-based masters degree. Providing graduate level food analysis experiments to CSUN students is therefore an important part of their graduate level education and experience.

Food Quality and Safety Using Analytical Tools

Food quality and safety are leading issues in today's food industry and economy, and consumers in developed countries have become more critical and demanding in regards to their food choices (Grunert, 2005). Price alone is not what drives consumers to buy a food product; they want to buy products that are safe and superior in quality, with

added value, such as possible health benefits. As consumers' demand for high quality and safe foods has increased, the development and application of analytical techniques and methods in the field of food science has grown as well (Garcia-Canas, Simo, Herrero, Ibanez, & Cifuentes, 2012).

Food analysis is one of the most important areas of study in food science because it covers a wide number of topics, such as verification of safety, authenticity, and quality of foods, determination of nutritional values, and detection of toxic molecules as well as beneficial compounds (Cifuentes, Dugo, & Fanali, 2013). The assessment of food quality and safety has been of extreme importance for regulatory agencies and the food industry, and the advancements in analytical technology have helped the modern food scientist tremendously (Cifuentes, 2009). Legislation is mainly focused on adulteration regarding substances that can or cannot be found in a particular food, as well as compounds that are potentially dangerous and have very low permissible limits (Cifuentes et al., 2013). The main goal of food analysis is to ensure food safety; in order to do this, laboratories have to be updated and must use modern analytical techniques (Garcia-Canas et al., 2012). Regulatory agencies, food chemists, quality control laboratories, and manufacturing facilities are all interested in more powerful and less expensive analytical instruments and methods. The globalization of food resulted in raw materials coming from around the world and this added a layer of complexity and challenges for food analysts who must develop new analytical methods and use the best available science and technology. As Cifuentes (2012) mentions, every analytical technique provides specific information, and all have advantages and disadvantages. A skilled food analyst must be able to select or create the best analytical method, or a combination of methods, to ensure reliable and

accurate results. The Association of Official Agricultural Chemists (AOAC International) provides official validated methods of analysis that are widely used by food analysts due to their internationally accepted accuracy and validity (Ruth, 2002). The AOAC Methods of Analysis database is in accordance with the International Organization for Standardization (ISO) 17025, which is being used as a gold standard in food analysis laboratories throughout the world. Two widely used methods of analysis are atomic spectroscopy such as AAS, and separation methods like HPLC. Both the AAS and HPLC will be discussed next as the experiments developed for this project use these two instruments to quantify for different compounds in foods, such as capsaicin, coumarin, and lead.

HPLC Background and Use in the Food Science Industry

The food industry uses a large variety of analytical methods to ensure the safety and quality of foods. Chromatography is among the most sensitive and widely used method due to its high selectivity (Di Stefano et al., 2012). Developed in the 1960s, HPLC is a high-pressure version of the classic liquid column chromatography (Reuhs & Rounds, 2010). The components of a HPLC system include a solvent delivery pump, a high-pressure column, a sample injection valve, a detector, and a computer that displays the results. The column contains very fine particles (stationary phase), and the solvent (mobile phase) is pushed at high pressure through the column, giving high-resolution separations. In normal-phase HPLC, the stationary phase is polar, and the mobile phase is a nonpolar solvent, such as hexane. The sample being analyzed has to dissolve in the mobile phase, so it has to be non-polar. This type of HPLC analysis is used for fat-soluble vitamins. In reversed-phase HPLC, the stationary phase is non-polar and the mobile

phase is polar, which is usually water mixed with methanol or acetonitrile. This method is the most widely used due to its versatility.

Capsaicinoids are a group of alkaloids that are responsible for the pungency of chili peppers (Batchelor & Jones, 2000). The main capsaicinoids present in peppers are capsaicin and dihydrocapsaicin, which are responsible for about 90% of the spiciness of peppers (Perucka & Oleszek, 2000). The most common analytical methods of quantifying capsaicinoids are gas chromatography (GC) and HPLC. Even though GC offers high sensitivity, it is considered inferior to HPLC due to the fact that it requires complex sample preparation, which is time consuming and costly (Chiang, 1986). In their teaching laboratory experiment, Batchelor and Jones (2000) aimed to determine three capsaicinoids in food products. The experiment was to be conducted at a reasonable price and within a four-hour time frame. Upon completion of the experiment, students would learn to perform simple extraction techniques, refine standard-making and sample-handling techniques, and understand the principle behind HPLC. In the methods section, ethanol (95%) was used for extraction, and the mobile phase for the HPLC reverse-phase run was composed of acetonitrile, water, and phosphoric acid. The authors concluded that various commercial hot sauces, ground chilies, and capsaicin-based arthritis creams could be used successfully in this experiment.

Another teaching experiment was developed by Betts (1999), in which students are asked to develop an HPLC method to separate and quantify capsaicins. The standard solution was made from a mixture of 65% capsaicin and 35% dihydrocapsaicin. Acetone was used for the extraction of capsaicin from peppers, and dehydrated ethanol was used to dilute the samples after extraction. The mobile phase used in this collectively

developed method was a mixture of 80:20 methanol and water. The author concluded that using this collective approach to develop a method provides pedagogical advantages because it teaches students to solve realistic problems using literature and instruments available. Gonzalez-Zamora et al. (2013) investigated the effect of temperature on the content of capsaicinoids in different varieties of peppers. Extraction was done with acetonitrile at 65° C for 20 minutes. The mobile phase for the HPLC quantitation was a mixture of 50:50 water:acetonitrile. Detection was set at 222 nm and 280 nm. The authors concluded that there was a great variability in capsaicinoids and temperature had a significant effect on the type and total capsaicinoids in peppers.

Coumarin is a naturally occurring substance, which is found in a variety of plants such as green tea and chicory, and in essential oils such as bark oil, peppermint oil, and lavender oil (Lungarini, Aureli, & Coni, 2008). The main source of coumarin in the diet, however, is cinnamon. There are two main types of cinnamon: *Cinnamomum verum*, also known as true cinnamon, which contains only a trace amount of coumarin (about 0.004%) and is more expensive; and *Cinnamomum cassia*, which contains up to 1% coumarin. This is the most commonly used type of cinnamon (Blahova & Svobodova, 2012). Coumarin is also known to cause liver and kidney damage in rats, and in the 1980s it was considered a possible genotoxic carcinogen in humans (Sproll, Ruge, Andlauer, Godelmann, & Lachenmeier, 2008). This caused the European Union (EU) to set specific maximum levels for coumarin at 2 mg/kg in most foods, except for special caramels and alcoholic beverages, which had a 10 mg/kg permissible maximum level. However, new data showed that coumarin is non-genotoxic in humans, though a subgroup of individuals has a high sensitivity to coumarin and its hepatotoxic effect (Ballin & Sørensen, 2014). In

2008, the EU established new maximum limits for coumarin at 50 mg/kg in bakery products, 20 mg/kg in breakfast cereals, and 5 mg/kg in desserts. It is also important to mention that coumarin should not be added to any food product; it should only come from natural sources.

Quantification of coumarin in foods can be performed through different methods of analysis; however, HPLC quantification is the most reliable and widely used method (Ballin & Sørensen, 2014). The food samples are usually extracted with either methanol, ethanol, or hexane. The mobile phase used can be a mixture of orthophosphoric acid:methanol:acetonitrile (80:10:10) for an isocratic elution or acetonitrile:methanol (1:2) (Blahova & Svobodova, 2012; Lungarini et al., 2008; Sproll et al., 2008). There seems to be a consensus among the results of the above-mentioned studies in the fact that bakery products, especially cookies, contain higher than permissible amounts of coumarin. This might be due to the fact that bakeries are not aware of the coumarin regulations, or they do not know how to decrease their over-the-limit coumarin levels.

AAS Background and Use in the Food Science Industry

Atomic spectroscopy became widely available in 1960s, which paved the way for tremendous advances in food analysis, nutrition, and toxicology (Miller & Rutzke, 2010). Theoretically, all elements could be analyzed by atomic absorption. In reality, AAS is used mainly for determining the quantities of mineral elements. There are two types of atomic absorption instruments: flame AAS and graphite furnace AAS. They both work on the same principle, except the atomization process is different. In both flame AAS and graphite furnace AAS, the sample to be tested needs to be in solution so that it can be converted to vapors. The sample is nebulized and then burned in a flame. The sample is

decomposed by high temperature flame and atoms are formed. A beam of radiation at specific wavelength is passed through the flame, which detects the quantity of that specific atom being analyzed. Each element has a unique cathode lamp that emits radiation at a specific wavelength for that element. For this project, lead was one of the elements that was available for analysis because a lead cathode lamp was available.

Lead exposure is a serious public health issue, especially the sub-lethal exposure from contaminated foods (Ndung'u, Hibdon, & Flegal, 2004). Among the foods that contain high amounts of lead is vinegar. This might be due to the grapes the vinegar is made of; lead can have an endogenous origin, or it can come from contamination during the production process. Ndung'u, Hibdon, & Flegal, (2004) recommend that the analysis of lead in vinegar be conducted by graphite furnace AAS, using a magnesium nitrate/ammonium phosphate modifier and digestion of the samples with nitric acid. Their results indicated that lead concentrations in vinegar vary from less than 10 μg/L to more than 300 μg/L. The highest lead concentrations were found in balsamic vinegar, probably due to contamination from metal fittings during barrel aging.

As the food industry tries to keep up with consumer and government demands for safe and high quality foods, the Food Science programs have the responsibility to educate and prepare highly skilled professionals. The IFT has contributed greatly to the development of educational standards in food science in the United States. Understanding the principles behind important analytical tools such as AAS and HPLC and being able to quantify different compounds with analytical precision is of uttermost importance for any modern food scientist. Mastering such technical skills will help the CSUN Food Science

students be more competitive in the job market and be ready to solve real life problems in food science applications.

CHAPTER III

METHODOLOGY

This chapter will discuss the process of selecting, designing, and testing the three Food Chemistry and Analysis experiments. After consultation with the CSUN Food Science coordinator, Dr. Claudia Fajardo-Lira, it was decided to develop three Food Chemistry and Analysis experiments that will be taught in a graduate Food Chemistry class. Evaluation criteria for selecting the three experiments included 1) analytical instrumentation availability, 2) chemical reagents and standards availability and cost, 3) time necessary to run each experiment, and 4) relevance of the food chemistry and analysis principles taught in the graduate class.

The analytical instruments available included one HPLC and one graphite furnace AAS. The HPLC was not in working condition. However, after several troubleshooting problems were solved, and new software was installed, the instrument was running. Several purging cycles and a calibration were performed before any experiment was carried out. The graphite furnace AAS was not in working condition either. After several attempts to run a water sample, two electrical components broke and had to be replaced. After the successful replacement of the faulty components, the AAS started giving accurate absorption readings, meaning that the absorption levels increased with increasing concentration. With both the HPLC and AAS in working condition, the writing and testing of the proposed experiments took place.

After a careful literature review, and based on the availability of analytical equipment in the food chemistry lab of the Family and Consumer Sciences at CSUN, the following three experiments were chosen:

1. Determination of Capsaicinoids in Hot Peppers by HPLC
2. Determination of Coumarin Levels in Ground Cinnamon by HPLC
3. Determination of Lead in Vinegar by AAS

Each experiment would include the following sections:

- *Objective:* A brief statement describing the purpose of the experiment.
- *Background Principles and Theory:* A detailed explanation of the principles behind the analytical technique.
- *Reagents and Materials:* A comprehensive list of all the equipment, chemical reagents, and testing samples used in the experiment.
- *Procedures:* Step-by-step detailed instructions on how to properly run the experiment.
- *Handling of Waste:* A clear statement instructing the students on how to safely dispose of the chemical wastes during and after the experiment.
- *Results and Calculations:* Brief statement guiding the students on how to compute the final results. The instructor, if needed, should provide additional explanations or examples to the students.
- *Discussion:* Students' interpretation of the results and well thought explanation that relates the principles of the analytical method with the obtained results.
- *Questions:* Two to five questions designed to improve students' grasp of the analytical principles used in the experiment. Challenge students' critical thinking by asking them to apply the learned analytical methods in other food science analytical case studies.
- *References:* A list of all the sources used in writing the laboratory experiment.

Each section of the laboratory experiments was very clearly and concisely written to ensure that students have a worthwhile educational experience in the lab. The experimental methods of analysis were created based on published literature, and adapted to the available instruments and chemical reagents.

Each experiment was tested three times to ensure that procedure instructions are clear and easy to follow. In addition, an Instructor Notes sheet was developed to aid the instructor in becoming more familiar with the details of the analytical methods used. Appendix D contains all the Material Safety Data Sheets (MSDS) for the chemical reagents used in the experiments.

CHAPTER IV

RESULTS

The purpose of this project was to create three Food Chemistry and Analysis experiments that expose CSUN Food Science students to the principles and laboratory use of analytical instruments such as HPLC and AAS. The developed experiments were tested in SQ 134 at CSUN. Appendix A contains the three experiments as they will be provided to the students. A set of trials of the experiments, including analytical results, standard curve generation, and unknown calculation are included in Appendix B. The mobile phase used to determine capsaicinoids by HPLC was 65:35:1 water:methanol:acetic acid. For coumarin, the mobile phase used was 25:75 water:methanol. The capsaicin peak was resolved at 4.2 minutes, dihydrocapsaicin peak was resolved at 3.2 minutes, and the coumarin peak was detected at 8 minutes.

In order to create a sustainable educational practice, it is also important to provide instructors with specific guidelines pertaining to each experiment. Appendix C contains instructor notes that would guide them on how to operate the HPLC and AAS instruments. This appendix also contains guidelines on how to assist students with calculations for the experiments and instructions for the laboratory technicians, which will help them prepare for each experiment. It is important for both the instructors and the students to be familiar with the reagents used in each experiment, so the MSDS sheets of all the chemical reagents used are included in Appendix D.

CHAPTER V

DISCUSSION

The purpose of this project was to develop and test three Food Chemistry and Analysis experiments, which expose CSUN Food Science students to the principles and laboratory use of analytical instruments such as HPLC and AAS. Determination of capsaicinoids in hot peppers and determination of coumarin levels in ground cinnamon by HPLC were tested with an isocratic method using a 65:35:1 mixture of water:methanol:acetic acid and 25:75 water methanol respectively. Each experiment took 2 class sessions to complete, the first class period was used to extract the unknown sample and prepare the standard solutions, and the second class period was used to run the HPLC analysis. All the chromatograph peaks were resolved in a timely manner. Determination of lead in vinegar took only one laboratory session due to the short AAS detection time. The white wine vinegar used to quantify for lead content was not the ideal sample to test because it did not have a high lead concentration (54.4 ppb). A recommendation for improving this experiment would be to use balsamic vinegar or red wine vinegar, which has a higher lead content, and to prepare the sample by digesting it and using a modifier. Even though the methods used in these three experiments differ from the methods found in the literature (Perucka & Oleszek, 2000; Betts, 1999; Blahova & Svobodova, 2012; Lungarini et al., 2008; Sproll et al., 2008), they produced feasible results and saved money and time for the Food Science Department.

Discussion of the Findings and Modifications

Discussion of the Expert Evaluation

There were significant changes made to the method of analysis by HPLC in order to accommodate and synchronize with the available reagents and instrumentation in the Food Science laboratory at CSUN. Further, due to time constrains, an expert evaluation was not possible for this project. However, it is crucial that the experiments are evaluated to ensure educational quality and value. Experts in Food Science and Nutrition field should be utilized to evaluate the three proposed Food Chemistry and Analysis experiments. The experts would be expected to read the experiments and conduct each method of analysis in order to be able to propose modifications if necessary. The main criteria by which the three experts would be chosen to evaluate the Food Chemistry and Analysis experiments is experience in the food industry and familiarity with Food Chemistry and Analysis laboratory work. They should be emailed a copy of the experiments and allowed 3 weeks to evaluate it and propose changes. They would be asked open-ended questions such as "What changes would you make in the *Procedures* section?". Closed-ended questions would also be asked, such as "Is the *Background* section clear and informative?".

Discussion of the Target Population Evaluation

In order to produce high educational value experiments, the opinion and edits of students performing the experiments is also important. The target population for the evaluation would be the food science students who will perform the experiments during a Food Analysis class offered at CSUN.

A recommendation for a future project would be to develop and administer student surveys during the pilot run of the experiments. This will give valuable feedback on the educational value of the proposed experiments and will help instructors make adjustments to enhance student learning.

Implications

The three Food Chemistry and Analysis experiments in this project were developed to address the need for providing more up-to-date food analysis topics that will not only serve the students, but will also provide instructors with valuable teaching tools. The students will ultimately get the hands-on analytical laboratory experience that will help them build the skills needed to succeed in today's competitive job market.

Conclusion

The purpose of this project was to create and test three Food Analysis and Chemistry up-to-date experiments that would expose students to the use of important analytical tools and enhance their laboratory knowledge and skills. The two experiments using the HPLC machine expose the students to the details of sample preparation, standard curve generation, and running the instrument. The AAS experiment provides students with a valuable learning experience on how to accurately detect elements in minute amounts. The proposed experiments will bring the Food Science program at CSUN closer to the IFT Education Standards and will provide students with the necessary analytical skills to succeed as a food scientist.

References

Arnold, R. G., Berry, R. E., Bradley, E., Clark, W. L., Clausi, A. S., Denton, A. E.,…Somogyi, L. P. (2000). *A century of food science*. Retrieved from http://www.ift.org/~/media/PDF/A_Century_of_Food_Science_web.pdf

Ballin, N., & Sorensen, A. (2014). Coumarin content in cinnamon containing food products on the danish market. *Food Control*, *38*, 198-203. doi: 10.1016/j.foodcont.2013.10.014

Batchelor, J., & Jones, B. (2000). Determination of the scoville heat value for hot sauces and chilies: An hplc experiment. *Journal of Chemical Education*, *77*(2), 266-267. doi: 10.1021/ed077p266

Betts, T. (1999). Pungency quantitation of hot pepper sauces using hplc. *Journal of Chemical Education*, *76*(2), 240-244. doi: 10.1021/ed076p240

Blahova, J., & Svobodova, Z. (2012). Assessment of coumarin levels in ground cinnamon available in the czech retail market. *Scientific World Journal*, *2012*, 263851-263854. doi:10.1100/2012/263851

Chiang, G. (1986). Hplc analysis of capsaicins and simultaneous determination of capsaicins and piperine by hplc-ecd and uv. *Journal of Food Science*, *51*(2), 499-503. doi: 10.1111/j.1365-2621.1986.tb11165.x

Chikthimah, N., & Floros, J. (2007). Challenges to food science in the U.S. *Journal of Food Technology, 61*(3), 38-44. Retrieved from https://illiad.csun.edu/illiad/illiad.dll?Action=10&Form=75&Value=279211

Cifuentes, A. (2009). Food analysis and foodomics. *Journal of Chromatography A*, *1216*(43), 7109. doi:10.1016/j.chroma.2009.09.018

Cifuentes, A. (2012). Food analysis: Present, future, and foodomics. *ISRN Analytical Chemistry*, *2012,* 1-15. doi: 10.5402/2012/801607

Cifuentes, A., Dugo, P., & Fanali, S. (2013). Advances in food analysis. *Journal of Chromatography A*, *1313*, 1. doi: 10.1016/j.chroma.2013.09.024

Di Stefano, V., Avellone, G., Bongiorno, D., Cunsolo, V., Muccilli, V., Sforza, S.,…Vékey, K. (2012). Applications of liquid chromatography-mass spectrometry for food analysis. *Journal of Chromatography A*, *1259*, 74-85. doi: 10.1016/j.chroma.2012.04.023

Garcia-Canas, V., Simo, C., Herrero, M., Ibanez, E., & Cifuentes, A. (2012). Present and future challenges in food analysis: Foodomics. *Analytical Chemistry*, *84*(23), 10150-10159. doi: 10.1021/ac301680q

Grunert, K. (2005). Food quality and safety: Consumer perception and demand. *European Review of Agricultural Economics*, *32*(3), 369-391. doi:10.1093/eurrag/jbi011

Gonzalez-Zamora, A., Sierra-Campos, E., Luna-Ortega, J., Perez-Morales, R., Ortiz, J., & Garcia-Hernandez, J. (2013). Characterization of different capsicum varieties by evaluation of their capsaicinoids content by high performance liquid chromatography, determination of pungency and effect of high temperature. *Molecules. 18*(11), 13471-13486. doi: 10.3390/molecules181113471

Hartel, R. (2002). Core competencies in food science: Background information on the development of the ift education standards. *Journal of Food Science Education*, *1*(1), 3-5. doi: 10.1111/j.1541-4329.2002.tb00003.x

Hartel, R. (2006). 2001 ift education standards: A 5‐year perspective. *Journal of Food Science Education*, *5*(4), 65-69. doi: 10.1111/j.1541-4329.2006.00015.x

Iwaoka, W. (2011). Introduction to the ift 2011 resource guide for approval and re‐approval of undergraduate food science programs. *Journal of Food Science Education*, *10*(4), 54-56. doi: 10.1111/j.1541-4329.2011.00130.x

Lungarini, S., Aureli, F., & Coni, E. (2008). Coumarin and cinnamaldehyde in cinnamon marketed in italy: A natural chemical hazard?. *Food Additives & Contaminants: Part a*, *25*(11), 1297. doi: 10.1080/02652030802105274

Miller, D. D., Rutzke, M. A. (2010). Atomic absorption spectroscopy, atomic emission spectroscopy, and inductively coupled plasma-mass spectrometry. In Nielsen, S. S. (Ed.), *Food analysis* (pp. 421-441). West Lafayette, IN, USA: Springer.

Ndung'u, K. , Hibdon, S. , & Flegal, A. (2004). Determination of lead in vinegar by icp-ms and gfaas: Evaluation of different sample preparation procedures. *Talanta*, *64*(1), 258-263. doi: 10.1016/j.talanta.2004.02.017

Perucka, I., & Oleszek, W. (2000). Extraction and determination of capsaicinoids in fruit of hot pepper capsicum annuum l. by spectrophotometry and high-performance liquid chromatography. *Food Chemistry*, *71*(2), 287-291. Retrieved from http://ac.els-cdn.com.libproxy.csun.edu/S0308814600001539/1-s2.0-S0308814600001539-main.pdf?_tid=563b4a3a-d26d-11e4-962b-00000aacb35d&acdnat=1427232956_7496a2bfbfbf258a3530f690ebd17722

Reuhs, B. L., Rounds, M. (2010). High-performance liquid chromatography. In Nielsen, S. S. (Ed.), *Food analysis* (pp. 499-537). West Lafayette, IN, USA: Springer.

Roberts, A., Robbins, J., McLandsborough, L., & Wiedmann, M. (2010). A 10‐year review of the food science summer scholars program: A model for research training and for recruiting undergraduate students into graduate programs and careers in food science. *Journal of Food Science Education*, *9*(4), 98-105. doi: 10.1111/j.1541-4329.2010.00103.x

Ruth, L. (2002). Standardizing food analysis. *Analytical Chemistry*, *74*(3), 67A. doi: 10.1021/ac021933b

Sproll, C., Ruge, W., Andlauer, C., Godelmann, R., & Lachenmeier, D. (2008). Hplc analysis and safety assessment of coumarin in foods. *Food Chemistry*, *109*(2), 462-469. doi:10.1016/j.foodchem.2007.12.068

Weller, D., Robbins, J., Elmore, A., & Wiedmann, M. (2015). Master of professional studies in agriculture and life sciences offered through the field of food science and technology at cornell university: A model for the development of a course‐based graduate degree in food science and technology. *Journal of Food Science Education*, *14*(1), 10-17. doi: 10.1111/1541-4329.12047

APPENDIX A

This Appendix contains the following experiments:

1. Determination of Capsaicinoids in Hot Peppers by HPLC
2. Determination of Coumarin Levels in Ground Cinnamon by HPLC
3. Determination of Lead in Vinegar by AAS

Determination of Capsaicinoids in Hot Peppers by HPLC

Objective

The purpose of this experiment is to determine capsaicin and dihydrocapsaicin content in hot peppers using an isocratic high performance liquid chromatography.

Background

Liquid chromatography is a separation technique based on the affinity of an analyte between two liquid phases. One is permanently bonded to small solid support particles and then packed inside a stainless steel tube a few mm in diameter and a few cm in length. This forms the packed stationary phase column. Sometimes a short length of 'guard column' is added before the main column to trap substances that would be irreversibly retained by the main column and so prolong its lifespan. The other phase is the mobile phase, a mixture of liquids that sweep the analyte through the packed column. A high pressure pump is necessary to push the mobile phase through the column. Dissolved gases in the mobile phases can create bubbles inside the column and interfere with detection or elution and particles can block the tiny pores between support particles. As such, the solvents are commonly 'sparged' (degassed by bubbling He or N through them) and filtered.

Analytes interact with both the mobile phase and the stationary phase and establish equilibrium. Those analytes that spend longer dissolved in the stationary phase are delayed and emerge from the column later. The polarity of the stationary phase liquid and mobile phase liquids can be altered to enhance the separation. If the mobile phase remains constant during the separation it is termed *isoscratic* elution whereas if the mobile phase composition is changed during the separation it is termed *solvent*

programming or *gradient* elution. In this experiment, the stationary phase is mostly non-polar. The mobile phase is a mixture of water and methanol, a polar solvent. This combination of polarities is termed 'reverse phase' conditions.

Applications for HPLC cover wide-ranging areas, including biochemistry, environmental analysis, and food science. For this experiment, you will analyze peppers for their capsaicinoid content using isocratic high performance liquid chromatography HPLC. Capsaicinoids are the compounds responsible for the pungency (i.e., spiciness) of pepper fruits and their products. Capsaicin and dihydrocapsaicin comprise over 90% of the "heat" in peppers; their structures are shown below.

The degree of hotness of spicy foods is typically reported in Scoville Heat Units (SHU). The various capsaicin compounds have been assigned reference SHU values, corresponding to the pure component. The capsaicinoids in your samples will be separated based on their polarities using reversed-phase conditions and will be detected spectrophotometrically. By comparing the results for capsaicin standards to those for your samples, a quantitative measurement will be made, and using the reference SHU for each compound, the total Scoville Heat Value (SHV) will be calculated. Please be aware that although tables of SHU and SHV can be found on the internet, the broad ranges quoted are usually not accurate for this experiment.

Reagents and Materials

1. Schimadzu HPLC system (pump model LC10ATvp; system controller model SCL10Avp; autosampler model SIL10ADvp; detector model SPD10Avp)
2. Plastic pipette
3. 0.45 μm filter disk

4. Mortar and pestle
5. 50 mL centrifuge tube
6. HPLC grade water and methanol
7. Stock capsaicin solution (65% capsaicin and 35% dihydrocapsaicin)
8. Pepper sample (habanero or jalapeño)

Procedure

A. Unknown Preparation

1. Obtain a pepper (with a mass of at least 3 g) and remove the stem. Place it for 10 minutes in -80 ° C freezer. Crush the pepper under the hood using the mortar and pestle and grind until it becomes a fine powder. The capsaicin standard can cause skin and eye irritation and breathing difficulties. **Wear gloves and eye protection. Grind your peppers in the hood.**
2. Weigh a 50-mL centrifuge tube.
3. Place the powdered pepper into the tube and re-weigh. Calculate the mass of the tube.
4. Add 35.00 mL of methanol to the tube and shake on the orbital shaker for about 1 hour.
5. Refrigerate your labeled centrifuge tube until the next laboratory period. Reweigh the tube plus its contents. Calculate the mass of the solution.
6. Filter about 5 mL of the supernatant (the liquid) through a 0.45-μm filter. Dilute 5 mL of your unknown to 10 mL total volume with HPLC grade methanol to make a 50% concentration solution of your unknown.

7. Dilute 5 mL of your 50% unknown made in step 6 to 10 mL total volume with HPLC grade methanol to make a 25% concentration solution of your unknown.
8. Dilute 5 mL of your 25% unknown made in step 7 to 10 mL total volume with HPLC grade methanol to make a 12.5% concentration solution of your unknown. You should have three unknown solutions: unknown A = 50%, unknown B = 25% and unknown C = 12.5%.

B. Standard Preparation

A stock solution of capsaicin (in HPLC grade methanol) will be provided. Calculate the concentration of your stock solution in ppm ($d_{methanol}$ = 0.791 g/mL). Using successive dilutions, prepare four standards (in HPLC-grade methanol) between approximately 10 and 100 ppm. If necessary, store these solutions in a labeled beaker in the refrigerator until the next laboratory period.

C. Instrument Operation

1. Open the autosampler and load the 2-mL vials into the appropriate locations. Standards 1-4 should be loaded into positions 0-3 and your filtered unknown pepper samples in position 4-6.

2. Select single run, make sure the Capsaicin method is selected and the right vial number is introduced.

3. After the sample has finished running, select Reports -> View Area%. Record your area percentage for the capsaicin peak (at approximately 4.2 min) and dihydrocapsaicin peak at approximately 3.2 min).

4. If the peak areas of unknown a, b or c are larger than the most concentrated standard or smaller than the least concentrated standard you may discard this data (it will be outside

the range of the calibration curve you will make). If none of your unknowns lie within the maximum-minimum range of your standards, contact your instructor. Average the two SHU/SHV values if two unknowns are within your calibration curve maximum and minimum.

Handling of Waste

Discard your standard solutions and unknown solution in the designated bin labeled HPLC WASTE.

Results and Calculations

1. Prepare a calibration curve based on the capsaicin standards and another one based on the dihydrocapsaicin standards (Hint: divide the total ppm in each of your standards into ppm capsaicin and ppm dihydrocapsaicin and use these values to plot each calibration curve).
2. Report the capsaicin and dihydrocapsaicin peak areas, retention times and calculated concentrations in your pepper sample in ppm and (g capsaicinoid/g sample) based on the standard calibration curves. Don't forget to account for any unknown dilutions (50%, 25% and/ or 12.5%) you made.
3. Calculate the SHU for each capsaicinoid in your pepper on the basis of dry weight (assume 85% water content). The SHU for pure capsaicin and dihydrocapsaicin are 1.6×10^7 each. Multiply this factor by the capsaicinoid concentration (g/g dry weight) to yield the SHU.
4. Calculate the Scovile Heat Value for your pepper sample by adding the SHUs for capsaicin and dihydrocapsaicin.
5. Include with your report the standard calibration curves, a representative

chromatogram for the standards and the unknown.

Discussion

Discuss your results and any possible outliers (use Grubbs test).

Questions

1. Provide a rationale for the relative elution order of capsaicin and dihydrocapsaicin. Does this order make sense with regard to the chromatography conditions used?
2. Why must you always use filtered, high purity solvents in HPLC?
3. How does the ratio of capsaicin to dihydrocapsacin in your pepper sample compare to that in the standard?

Determination of Coumarin Levels in Ground Cinnamon by HPLC

Objective

The purpose of this experiment is to determine coumarin content in ground cinnamon using an isocratic high performance liquid chromatography.

Background

Liquid chromatography is a separation technique based on the affinity of an analyte between two phases (one polar and the other one non-polar). One is permanently bonded to small solid support particles and then packed inside a stainless steel tube a few mm in diameter and a few cm in length. This forms the packed stationary phase column. Sometimes a short length of 'guard column' is added before the main column to trap substances that would be irreversibly retained by the main column and so prolong its lifespan. The other phase is the mobile phase, a mixture of liquids that sweep the analyte through the packed column. A high pressure pump is necessary to push the mobile phase through the column. Dissolved gases in the mobile phases can create bubbles inside the column and interfere with detection or elution and particles can block the tiny pores between support particles. As such, the solvents are commonly 'sparged' (degassed by bubbling He or N through them) and filtered.

Analytes interact with both the mobile phase and the stationary phase and establish equilibrium. Those analytes that spend longer dissolved in the stationary phase are delayed and emerge from the column later. The polarity of the stationary phase liquid and mobile phase liquids can be altered to enhance the separation. If the mobile phase remains constant during the separation it is termed *isoscratic* elution whereas if the mobile phase composition is changed during the separation it is termed *solvent*

programming or *gradient* elution. In this experiment, the stationary phase is mostly non-polar. The mobile phase is a mixture of water and methanol, a polar solvent. This combination of polarities is termed 'reverse phase' conditions.

Applications for HPLC cover wide-ranging areas, including biochemistry, environmental analysis, and food science. For this experiment, you will analyze coumarin in ground cinnamon using isocratic high performance liquid chromatography HPLC. Coumarin is a naturally occurring substance, which is commonly found in cinnamon. Coumarin is also known to cause liver and kidney damage in rats, and in the 1980s it was considered a possible genotoxic carcinogen in humans.

Reagents and Materials

1. Schimadzu HPLC system (pump model LC10ATvp; system controller model SCL10Avp; autosampler model SIL10ADvp; detector model SPD10Avp)
2. Plastic pipette
3. 0.45 μm filter disk
4. 50 mL centrifuge tube
5. HPLC grade water and methanol
6. Stock coumarin solution
7. Ground cinnamon sample

Procedure

A. Unknown Preparation

1. Obtain ground 3 g of cinnamon.
2. Weigh a 50-mL centrifuge tube.
3. Place the powdered cinnamon into the tube and re-weigh. Calculate the mass of the tube.
4. Add 35.00 mL of methanol to the tube and shake on the orbital shaker for about 1 hour.
5. Refrigerate your labeled centrifuge tube until the next laboratory period. Reweigh the tube plus its contents. Calculate the mass of the solution.
6. Filter about 5 mL of the supernatant (the liquid) through a 0.45-μm filter. Dilute 5 mL of your unknown to 10 mL total volume with HPLC grade methanol to make a 50% concentration solution of your unknown.
7. Dilute 5 mL of your 50% unknown made in step 6 to 10 mL total volume with HPLC grade methanol to make a 25% concentration solution of your unknown.
8. Dilute 5 mL of your 25% unknown made in step 7 to 10 mL total volume with HPLC grade methanol to make a 12.5% concentration solution of your unknown. Your should have three unknown solutions: unknown A = 50%, unknown B = 25% and unknown C = 12.5%.

B. Standard Preparation

A stock solution of coumarin (in HPLC grade methanol) will be provided. Calculate the concentration of your stock solution in ppm ($d_{methanol}$ = 0.791 g/mL). Using successive dilutions, prepare four standards (in HPLC-grade methanol) between approximately 10

and 100 ppm. If necessary, store these solutions in a labeled beaker in the refrigerator until the next laboratory period.

C. Instrument Operation

1. Open the autosampler and load the 2-mL vials into the appropriate locations. Standards 1-4 should be loaded into positions 0-3 and your filtered unknown pepper samples in position 4-6.

2. Select single run, make sure the Coumarin method is selected and the right vial number is introduced.

3. After the sample has finished running, select Reports -> View Area%. Record your area percentage for the coumarin peak (around min 8).

Handling of Waste

Discard your standard solutions and unknown solution in the designated bin labeled HPLC WASTE.

Results and Calculations

6. Prepare a calibration curve based on the coumarin standards.
7. Report the coumarin peak areas, retention times and calculated concentrations in your cinnamon sample in ppm and (g coumarin/g sample) based on the standard calibration curves. Don't forget to account for any unknown dilutions (50%, 25% and/ or 12.5%) you made.

Questions

1. What are the permissible coumarin limits in foods in the United States and in Europe?

2. The mobile phase is composed of 65:35 water:methanol. How would the retention time change if the mobile phase would be 40:60 water:methanol?

Determination of Lead in Vinegar by Atomic Absorption

Objective

The purpose of this experiment is to determine lead content in vinegar using a graphite furnace atomic absorption spectrophotometer.

Background

Atomic absorption spectrophotometry (AAS) has become a routine method for the determination of many trace elements in a variety of sample matrices. The sample is vaporized and atomized by high temperatures, most simply by introduction into a flame. One of the limitations of flame AAS is the sensitivity of the method, partly because the sample spends only a short time in the light beam used for the absorbance measurement. However, the sensitivity of detection for most elements is significantly improved using a graphite furnace in place of the flame to atomize the sample. By vaporizing and atomizing inside a confined volume, the graphite furnace, and using Ar(g) to swirl the sample through the light beam many times before it finally escapes, the sensitivity increases.

In graphite furnace AAS, the liquid sample is placed on a small platform or in a small cup in the furnace and heated in a series of programmed heating steps to (1) dry, (2) ash (pyrolyze or char) and ultimately (3) atomize the sample. The low temperature drying step removes solvent, the ashing step decomposes and removes organic components from the matrix and finally, the high temperature atomization step vaporizes and atomizes the remaining sample.

Frequently, a matrix modifier is added to the sample prior to heating so that the chemical form of the analyte is controlled during the heating sequence. Although solid samples can

also be analyzed with the furnace, it is generally more desirable to dissolve such samples to minimize matrix effects. In this experiment, a sample of vinegar will be analyzed using graphite furnace AAS and the method of standard addition.

In the standard addition method, known quantities of lead standard solution are added to a solution containing the unknown. A series of successive additions produce an increase in the response of the instrument and the original amount of unknown can be determined by graphical or mathematical means. Standard addition is particularly useful when matrix effects are significant and simply preparing a solution of analyte without the matrix, as in a standard calibration curve for example, would produce serious errors.

One way to determine the amount of unknown in the original sample is by preparing a graph of the absorbance versus the amount of Pb added through the standard addition process. Extrapolation of the best-fit line to the standard addition data to zero absorbance yields the concentration of Pb in the original sample. The most straightforward way to calculate this value is to determine the equation for the best-fit line and solve for y = 0.

Equipment and reagents

1.Buck Scientific 220-AS AA spectrophotometer with graphite furnace

2. Plastic bottle

3. Plastic pipettes

4. Volumetric flasks

5. Milipore water

6. Pb standard solution

Procedure

1. Prepare a 100 ppm Pb stock solution.
2. From the 100 ppm stock solution prepare a 20 ppb standard solution. Store this solution in a plastic bottle and refrigerate it.
3. Prepare 6 solutions as follows*:

   *Use automatic pipettes and test tubes to prepare these solutions, cover with parafilm and invert the test tube a few times.

Sample	Composition
1	3 mL water* (blank)
2	1 mL unknown + 2 mL water
3	1 mL unknown + 1.2 mL water + 0.8 mL standard
4	1 mL unknown + 0.9 mL water + 1.1 mL standard
5	1 mL unknown + 0.5 mL water + 1.5 mL standard
6	1 mL unknown + 0 mL water + 2.0 mL standard

4. Load each sample into a plastic AAS tube
5. Press start. After the sample has been analyzed, record the absorbance number.

Handling of Waste

Dispose of all the Pb solutions in the designated bin labeled AAS WASTE.

Results and Calculations

1. Prepare a standard addition calibration curve in Excel by plotting the net peak area versus the concentration of the added Pb in the 3 mL sample for samples 2-6. Fit these data points with a linear least-squares line. Print this graph with the equation for the line.
2. Calculate the concentration of Pb in the vinegar from the equation for the standard addition calibration curve.

Questions

1. What are the likely sources of error in this experiment?

2. Which error is likely to be the largest? Assume good experimental technique. Describe how you might determine how much error is associated with this source.

Appendix B

This Appendix contains the trial experiments and results, including the standard curve and calculations of the unknown.

Determination of Capsaicinoids in Hot Peppers by HPLC

After the standards have been made, divide the total ppm values into ppm capsaicin and ppm dihydrocapsaicin according to the standard ratio of these components. For example, a 100 ppm standard would contain 65 ppm capsaicin (the standard is 65% capsaicin) and 35 ppm dihydrocapsaicin. Use these latter values for your x axes when plotting the calibration curves. In this way the ppm of the unknowns will be directly in ppm capsaicin or ppm dihydrocapsaicin as appropriate.

If the absorbance of the capsaicinoids were higher than the highest standard, you were told to dilute the original unknown sample. Let's assume you diluted it by a factor of 2 and that this is sufficient to bring your unknowns into the range of the calibration curves. For example, if you find from your calibration curve that you have 25 ppm of capsaicin and 10 ppm of dihydrocapsaicin, because of the dilution, your true levels will be 50 ppm of capsaicin and 20 ppm of dihydrocapsaicin.

Let's assume you have 40.00 g of total pepper + methanol solution. This means in your sample you would have:

$\underbrace{50\text{mg}/1000\text{ g}}_{50\text{ ppm}} \times 40.00\text{g} = 2.00$ mg of capsaicin and (by a similar calculation) 1.00 mg of dihydrocapsaicin (total = 3.00 mg).

Remember, the definition of parts per million is $\text{g}/10^6$ g or mg/1000 g.

Let's assume that the mass of (wet) pepper you used was 10.00 g. This means that, accounting for the mass of water in the pepper, the mass of dry pepper was 0.15 x 10.00 g = 1.50 g. Therefore, the g capsacin/g pepper is 2.00 $x10^{-3}$ g / 1.50 g = $1.33x10^{-3}$ g capsaicin / g pepper. A similar calculation for dihydrocapsaicin yields $6.67x10^{-4}$ g dihydrocapsaicin / g pepper.

Calculation of the SHU:

SHU capsaicin = $1.33x10^{-3}$ x $1.61x10^{7}$ = 21,467 and similarly the SHU dihydrocapsaicin = 10,733 and the total SHU (the SHV) is 32,200.

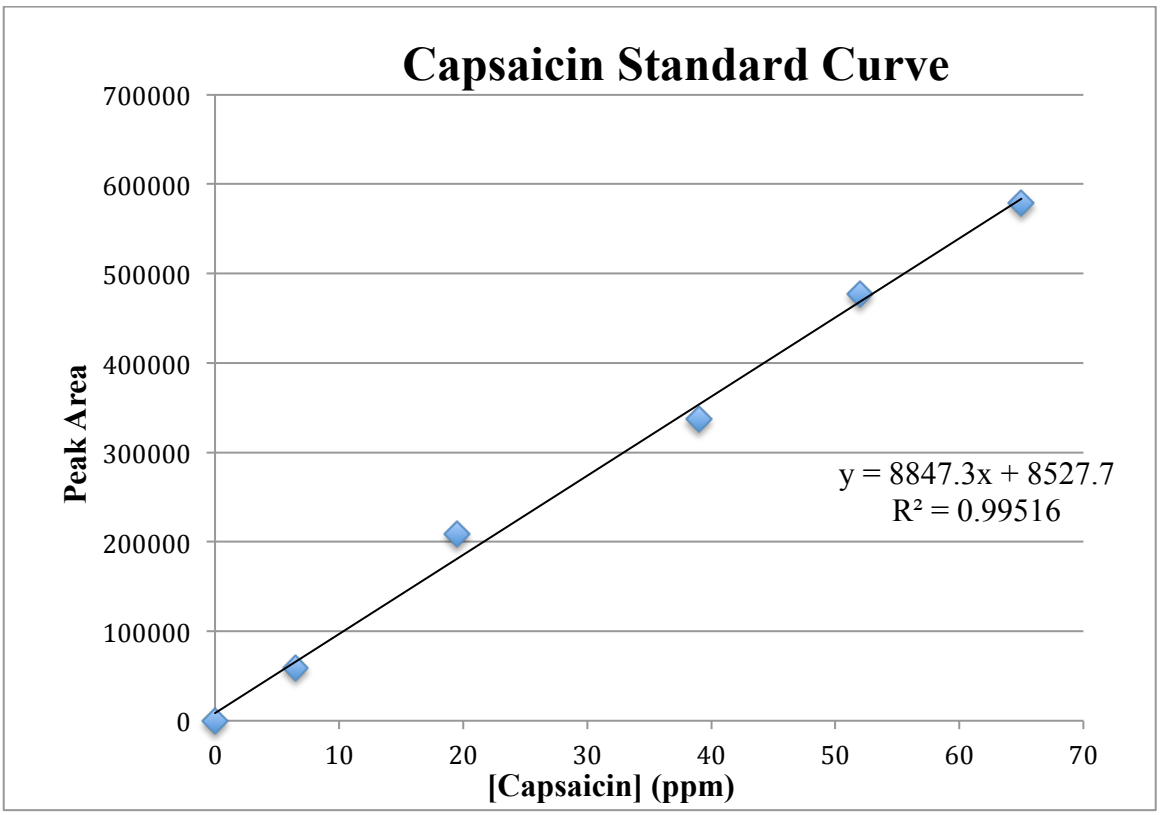
Note that this value is an example only: your value may or may not be close to this!

Note: Values for the SHU/SHV for Habanero peppers obtained from the internet are generally not reliable.

Results and Calculations

Capsaicin (~4.2 min.)	
ppm	Peak area
0	0
6.5	59197
19.5	209005
39	336974
52	477361
65	578845

Table 1. Capsaicin peak areas



Unknown had a peak area of 553389. The pepper sample weight was 4.3336 g.

553389 = 8847.3x + 8527.7

x = 61.58 ppm capsaicin

(61.58 mg/100 g) x 4.3336 g pepper sample = 2.67 mg capsaicin in wet sample.

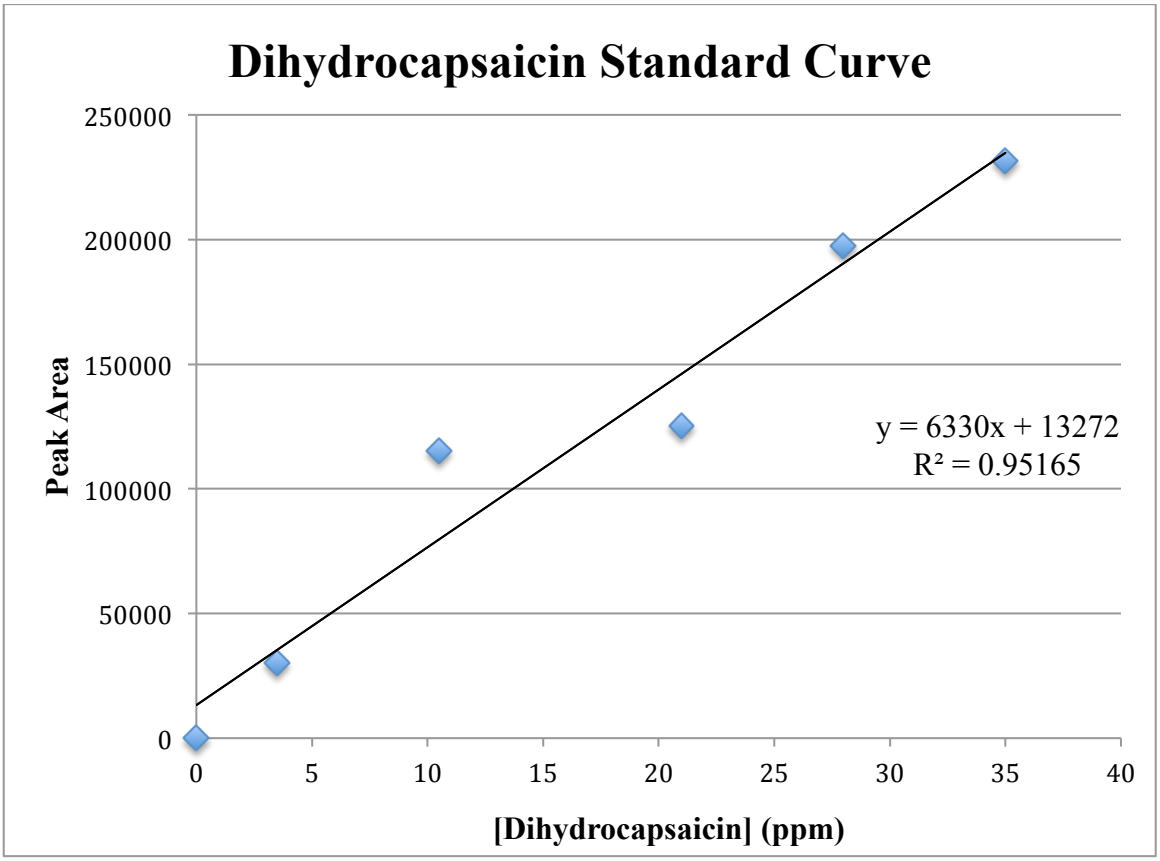
Account for the mass of water in pepper (approximately 85% wet mass, which means 15% dry mass): 0.15 x 4.3336 g = 0.65 g dry pepper mass.

2.67 mg capsaicin/0.65 g dry pepper → 0.00267 g capsaicin/0.65 g dry pepper → 0.0041 g capsaicin/g dry pepper.

SHU: 0.0041 x 1.61 x 10^7 = 66133.8.

Dihydrocapsaicin (~3.25 min.)	
ppm	Peak area
0	0
3.5	30201
10.5	115369
21	125177
28	197728
35	231499

Table 2. Dihydrocapsaicin peak areas



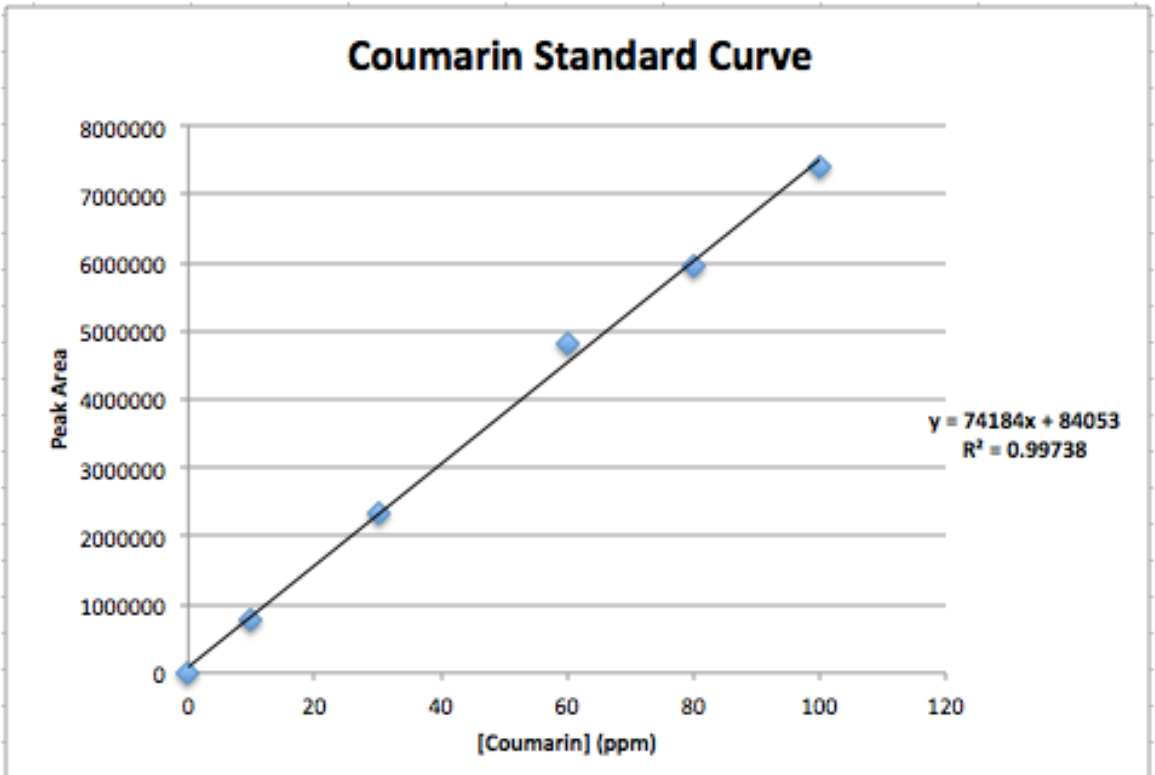
Dihydrocapsaicin peak area of the unknown was greater than the highest standard concentration (outside of standard curve), so determination of capsaicin is not possible. Recommendations to fix this problem would be to construct a different standard curve that would include de unknown reading or dilute the unknown until it falls on the standard curve.

Determination of Coumarin Levels in Ground Cinnamon by HPLC

Results

Concentration ppm	Peak area
0	0
10	778342
30	2349634
60	4824316
80	5934179
100	7389226

Table 3. Coumarin peak areas



Calculations

Coumarin was extracted from a 3.0717 g ground cinnamon using 35 mL methanol.

Unknown diluted 1:10 had a peak are of 1305632.

So, 1305632 = 74184x + 84053; x = 16.5 ppm.

16.5 ppm x 10 (dilution factor) = 165 ppm

We know that 1 ppm = 1 mg/L, so the sample had 165 mg/1000mL, however we had only 35 mL, so 165 mg/1000mL = x mg/35 mL → 5.7 mg in 35 ml, which means 5.7 mg in 3.0717 g cinnamon → 0.2 % coumarin.

Determination of Lead in Vinegar by Atomic Absorption

Assume the initial stock solution of Pb provided is 94.13 ppm or 94,130 ppb in Pb. This is diluted twice, first by taking 1 mL of the stock and diluting to 100 mL then by taking 4 mL of the resulting solution and diluting to 100 mL again. Hence the concentration of the final solution is

94.13 ppm x (1000 ppb/1 ppm) x (1 mL/100 mL) x (2 mL/100 mL) = 18.826 ppb

In the first (blank) solution the concentration of Pb = 0 ppb.

Sample	Composition
1	3 mL water* (blank)
2	1 mL unknown + 2 mL water
3	1 mL unknown + 1.2 mL water + 0.8 mL standard
4	1 mL unknown + 0.9 mL water + 1.1 mL standard
5	1 mL unknown + 0.5 mL water + 1.5 mL standard
6	1 mL unknown + 0 mL water + 2.0 mL standard

The second solution contains the unknown amount of Pb so the concentration of *added* Pb = 0. In the third solution (sample 3), 0.8 mL of the added standard is diluted to a total volume of 3 mL so the concentration of Pb added is

18.826 ppn x (0.8 mL/3 mL) = 5.02 ppb

In the fourth solution (sample 4), 1.1 mL of standard is diluted to 3 mL total. A similar calculation to that immediately above gives

18.825 ppb x (1.1 mL/3 mL) = 6.9 ppb

The other concentrations can be calculated similarly.

A graph of absorbance versus *added* Pb concentration is plotted and the slope and intercept determined. Since this is a standard addition experiment, the x-axis intercept gives the desired information about the unknown.

Let's assume that the equation for the best-fit line through the data, when [Pb] is in ppb, is given by A = 0.00961 x [Pb] + 0.2423 A. Solving this for y = 0, the x-axis intercept gives

y = 0.00961 x [Pb] + 0.2423 A

[Pb] = (*0.2423/0.00961) = 25.21 ppb *ignore the negative sign that is supposed to be there

Remember, each solution included 1 mL of unknown in the 3 mL total volume too. That means that the [Pb] = 25.21 ppb represents the diluted concentration. The original concentration must have been

$[Pb]_{undil}$ = 25.21 ppb x (3 mL/1 mL) = 75.64 ppb

Note that this value is an example only: your value may or may not be close to this!

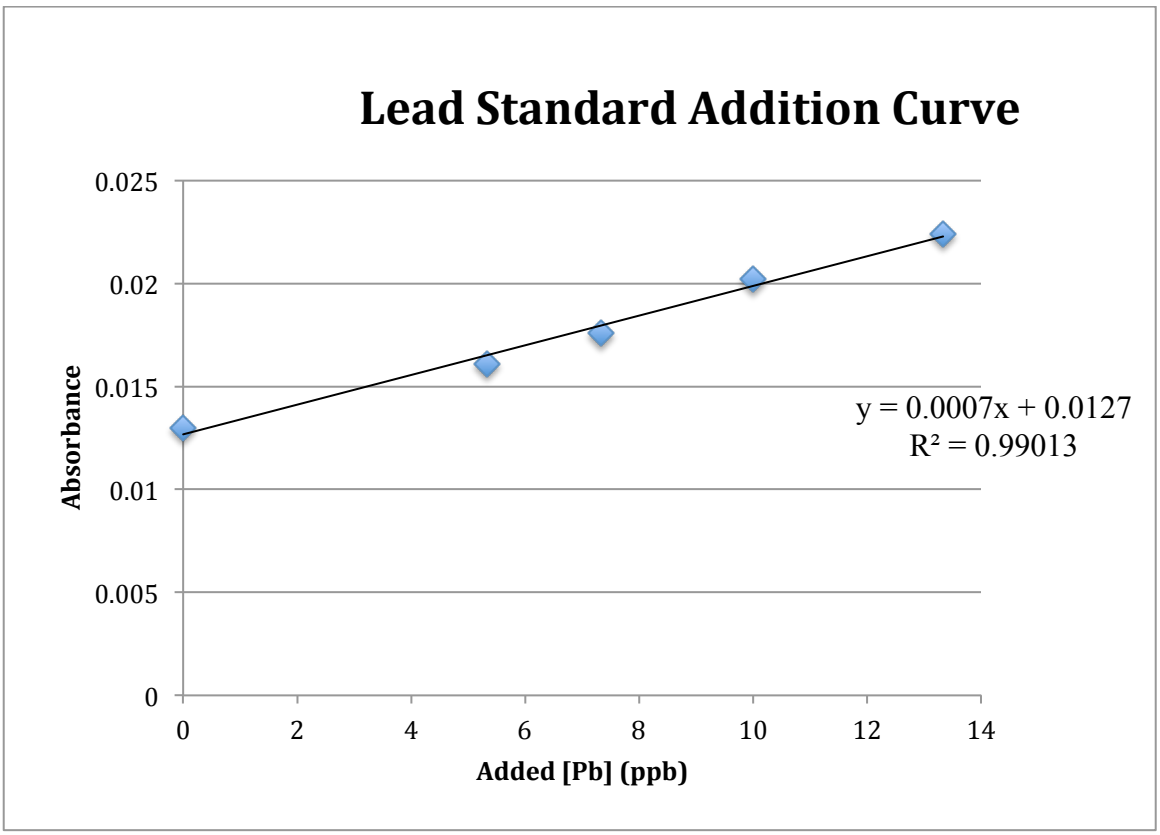
Results and Calculations

For this particular run, white wine vinegar was analyzed.

Sample 3 added [Pb]: 20 ppb x (0.8 mL/3 mL) = 5.33 ppb. The other samples' added [Pb] was calculated similarly.

[Pb] ppb	Absorbance
0	0.013
5.33	0.0161
7.33	0.0176
10	0.0202
13.33	0.0224

Table 4. Lead absorbance



0 = 0.0007x + 0.0127

x = 18.14 ppb

Adjust for dilution: 18.14 ppb x (3 mL/1 mL) = 54.42 ppb Pb in white wine vinegar.

Appendix C

This Appendix contains all the instructor's note necessary to smoothly conduct and teach each experiment.

HPLC Instrument Operation Instructor Notes

A. Instrument set-up before running samples.

1. Turn on all the HPLC components (pumps, detector, autosampler).
2. Open ClassVP 7.4 SP4 software.
3. Check the little green screen on the pump and make sure the maximum pressure is set to 5500 psi.
4. If the maximum pressure is set at a lower psi value, click Create/Modify Method.
5. Under the tab Pumps, type 5500 for the maximum pressure limits. Make sure the flow rate is set at 1.000 mL/min for both pump A and pump B.
6. Under the tab Autosampler, make sure that rack 2 is selected and press Detect Rack.
7. Under the tab SPD-10Avp (detector) make sure that the wavelength is set at 281 nm for Capsaicinoids experiment, and 278 for the Coumarin experiment. Make sure the run time is set at 6 minutes if running the Capsaicinoids experiment and 10 minutes if running the Coumarin experiment.
8. Press Apply at the bottom of the window, then press Download Method.
9. Check the screen of the pump to make sure the Maximum pressure is correct (5500 psi); check the autosampler screen to make sure that the correct rack is selected (Rack 2); check the UV-Vis detector to make sure that the correct wavelength is selected (281 nm for capsaicinoids, and 278 nm for coumarin).

B. Running a sample.

1. Press  (single run).

2. Enter sample ID (e.g. capsaicin std 10 ppm, capsaicin std 30 ppm, etc). Select the desired method (when analyzing capsaicin, select Capsaicin6.met, when analyzing coumarin, select coumarin 10).

3. Enter vial number (the slot in which you vial is, note that the numbering starts from 0). Make sure the injection volume is 25 µL.

4. Press start and wait for the sample to run.

5. After the sample has finished running, press  Stop Run.

6. To view or print results, select Reports, View or Print → Area %.

7. The results cannot be saved, so make sure you let the students know that they have to either write in their notebook the Area or print their results.

Furnace AAS Instrument Operation Instructor Notes

A. Settings for Lead Analysis

1. Make sure the graphite tube is uncoated and in good condition. If the graphite tube needs to be changed, follow instruction from the Instruction Manual (Section 4.0).
2. Set the correct parameters for determination of lead. The wavelength to be set is 283.3 nm and the slit should be 0.2 nm.
3. Make sure the lead lamp is in place. If not, you have to set it in place. Rotate lamp 3 in place. Press BKGD → Select to go to the next lamp. Press Lib and by using the arrows buttons, select Pb-Furn 283.2 Meth: Graphite Furnace. Once the Pb method is selected in Library window, press 2 to load this analysis from the library. Press Enter. Now you should have Active Analysis Lamp 3 Name: Pb-Furn3 – 283.2.

B. Running a Sample

1. Make sure the printer is on.
2. Press READ
3. Select 1 for First Sample
4. Press ENTER.
5. Make sure Absorbance results are displayed. If not, press CNTLS → Display Results → Abs-Emission.

Using Parts-Per-Million And Parts-Per-Billion

Some experiments in this project use concentrations in parts-per-million (ppm) and parts-per-billion (ppb). These are convenient units when solutions are very dilute. The definitions of ppm and ppb are:

$$1\text{ppm} = 1\text{ g}/1\text{x}10^6\text{ g} \qquad 1\text{ppb} = 1\text{ g}/1\text{x}10^9\text{ g}$$

These are not very useful definitions in themselves but they allow derivation of other expressions. Remember, for a dilute aqueous solution at room temperature, the density is almost exactly 1.00 g/mL or 1000 g/L.

1 ppm = $1\text{ g}/1\text{x}10^6\text{ g} = 1\text{ g}/1\text{x}10^6\text{ mL} = 1\text{ g}/1\text{x}10^3\text{ L}$ = **1 mg/L**

1 ppb = $1\text{ g}/1\text{x }10^9\text{ g} = 1\text{ g}/1\text{ x }10^9\text{ mL} = 1\text{ g}/\ 1\text{x}10^6\text{ L}$ = **1 µg/L**

When the solution is not aqueous, density must be taken into account or, better still, record the mass of the solution rather than its volume in your experimental data.

Example (1):

How many g of analyte are there in 12.00 mL of a 314 ppm solution?

In 1.00 L of solution there would be 314 mg of analyte. But we only have 12.00 mL of solution so the mass of analyte is:

314 mg/1 L = 314 mg/ 1000 mL = 314 µg/1 mL

Mass in 12.00 mL: (314 µg/1 mL) x 12 mL = 0.00377 g

Example (2):

What is the concentration (in ppb) of a solution that contains 0.00233 g of analyte in 980 mL of solution of density 0.920 g/mL?

The mass of solution is:

Mass = Volume x (Mass/Volume) = 980 mL x (0.920 g/1 mL) = 901.6 g

The concentration is:

Concentration = (0.00233 g/901.6 g) x $\underbrace{(1 \text{ ppb}/1\text{x}10^{-9} \text{ g})}_{\text{ppb conversion}}$ = 2580 ppb

Making Serial Dilutions

During the experiments in this project you will prepare a series of solutions of known concentration (**standards**) from one provided solution of known concentration (**stock solution**). There are several ways to prepare these solutions but perhaps the most efficient way is to perform a serial dilution; this means using one solution to prepare the next, more dilute solution. For example, you are asked to prepare at least 10 mL of three solutions between 100 and 1 ppm from a stock solution of 400 ppm.

Step one: To make the first (most concentrated) solution you will take 5 mL of the stock solution to make 25-mL of the first solution (you will use some of it later to prepare the others so you will make more than you need).

$M_1 \text{ X } V_1 = M_2 \text{ X } V_2$ M_1 = 400 ppm, M_2 = ? ppm, V_1 = 5 mL, V_2 = 25 mL

$M_2 = (M_1 \text{ x } V_1)/V_2$ = (400 ppm x 5 mL)/25 mL = 80 ppm

Although it might be tempting to try to make a 100 ppm solution, as requested, it simply is not as convenient to make given the stock solution and standard glassware. The quantity 5 mL was generated by trying various values in the equation until a reasonable M_2 concentration (between 1 and 100 ppm) resulted. Make sure this value is a convenient one (5-mL pipettes are standard but 4.275 mL pipettes are not!)

Step two: To make a second (less concentrated) solution, take 10 mL of the first solution and make 25-mL of new solution (use some of this solution later to prepare the last solution so make more than 10 mL).

$M_1 X V_1 = M_2 X V_2$ $M_1 = 80$ ppm, $M_2 = ?$ ppm, $V_1 = 10$ mL, $V_2 = 25$ mL

$M_2 = (M_1 \times V_1)/V_2 = (80 \text{ ppm} \times 10 \text{ mL})/25 \text{ mL} = 32 \text{ ppm}$

Step three: To make the final (least concentrated) solution, take 1 mL of the second solution and make 10-mL (use some of this solution later to prepare the last solution so make more than 10 mL).

$M_1 X V_1 = M_2 X V_2$ $M_1 = 32$ ppm, $M_2 = ?$ ppm, $V_1 = 1$ mL, $V_2 = 10$ mL

$M_2 = (M_1 \times V_1)/V_2 = (32 \text{ ppm} \times 1 \text{ mL})/10 \text{ mL} = 3.2 \text{ ppm}$

The final volumes you have are 15 mL of the 80 ppm solution, 15 mL of the 32 ppm solution and 10 mL of the 3.2 ppm solution.

Instructions for Laboratory Technician

Determination of Capsaicinoids in Hot Peppers by HPLC

Standard Preparation

1. Weigh about 0.1 g capsaicin and dilute in 100 mL HPLC grade methanol.
2. Calculate the exact concentration of capsaicin stock solution.

   Example: Let's say you weigh 0.1296 g capsaicin.

   Density of methanol 0.791 g/mL.

   100 mL solution x (0.791 g/mL) = 79.1 g (mass of solution)

   (0.1296 g/79.1 g) x (1 ppm/1 x 10-6 g) = 0.0016384 → 1638.4 ppm (concentration of standard stock solution). Make sure you store this solution in the refrigerator.
3. From this stock solution make a 100 ppm standard solution of capsaicin (use $M_1V_1 = M_2V_2$).
4. Prepare a dihydrocapsaicin standard solution of 100 ppm using the technique used for capsaicin.
5. Make a final standard of 65% capsaicin and 35% dihydrocapsaicin.

Determination of Coumarin Levels in Ground Cinnamon by HPLC

Standard Preparation

1. Weight about 0.1 g of coumarin and dilute to 100 mL with HPLC grade methanol.
2. Calculate the ppm of this initial stock solution and provide it to the instructor.

Determination of Lead in Vinegar by AAS

Standard Preparation

1. From the 1000 ppm Pb stock solution stored in the refrigerator prepare a 100 ppm solution to give to the studnets.
2. Make 100 ppm solution by diluting 10 mL of 1000 ppm lead standard solution to 100 mL with milipore water.

Appendix D

This final Appendix contains the MSDS sheets of all the reagents used in the proposed experiments.

Fisher Scientific

Part of Thermo Fisher Scientific

Material Safety Data Sheet

Creation Date 26-Jan-2010 **Revision Date** 28-Dec-2011 **Revision Number** 2

1. PRODUCT AND COMPANY IDENTIFICATION

Product Name **Water**

Cat No. : **W5-1; W5-4; W5N1-19; W5N2-19; W5SK-1; W5SK-4**

Synonyms No information available

Recommended Use Laboratory chemicals

Company
Fisher Scientific
One Reagent Lane
Fair Lawn, NJ 07410
Tel: (201) 796-7100

Emergency Telephone Number
CHEMTREC®, Inside the USA: 800-424-9300
CHEMTREC®, Outside the USA: 001-703-527-3887

2. HAZARDS IDENTIFICATION

Emergency Overview

Low hazard for usual industrial or commercial handling. Handle in accordance with good industrial hygiene and safety practice.

Appearance Clear, Colorless **Physical State** Liquid **Odor** Odorless

Target Organs None known

Potential Health Effects

Acute Effects
Principle Routes of Exposure

Eyes Non-irritating during normal use.
Skin Non-irritating during normal use.
Inhalation Not an expected route of exposure. Low hazard for usual industrial or commercial handling.
Ingestion Low hazard for usual industrial or commercial handling.

Chronic Effects
None known

Aggravated Medical Conditions No information available.

3. COMPOSITION/INFORMATION ON INGREDIENTS

Component	CAS-No	Weight %
Water	7732-18-5	100

4. FIRST AID MEASURES

Eye Contact Rinse immediately with plenty of water, also under the eyelids, for at least 15 minutes. Obtain medical attention.

Skin Contact Wash off immediately with plenty of water for at least 15 minutes. Get medical attention immediately if symptoms occur.

Inhalation Move to fresh air. Get medical attention immediately if symptoms occur.

Ingestion Clean mouth with water and drink afterwards plenty of water. Get medical attention if symptoms occur.

Notes to Physician Treat symptomatically

5. FIRE-FIGHTING MEASURES

Flash Point Not applicable
Method - No information available

Autoignition Temperature No information available

Explosion Limits
Upper No data available
Lower No data available

Suitable Extinguishing Media Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

Unsuitable Extinguishing Media No information available

Hazardous Combustion Products No information available.

Sensitivity to Mechanical Impact No information available
Sensitivity to Static Discharge No information available

Specific Hazards Arising from the Chemical
None known.

Protective Equipment and Precautions for Firefighters
As in any fire, wear self-contained breathing apparatus pressure-demand, MSHA/NIOSH (approved or equivalent) and full protective gear.

NFPA **Health** 0 **Flammability** 0 **Instability** 0 **Physical hazards** N/A

6. ACCIDENTAL RELEASE MEASURES

Personal Precautions Ensure adequate ventilation. Use personal protective equipment. Avoid dust formation.

Environmental Precautions Should not be released into the environment. See Section 12 for additional ecological information.

Methods for Containment and Clean Up Sweep up or vacuum up spillage and collect in suitable container for disposal. Avoid dust formation.

7. HANDLING AND STORAGE

Handling Wear personal protective equipment. Ensure adequate ventilation. Avoid contact with skin, eyes and clothing. Avoid ingestion and inhalation. Avoid dust formation.

Storage Keep containers tightly closed in a dry, cool and well-ventilated place.

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Engineering Measures None under normal use conditions.

Exposure Guidelines This product does not contain any hazardous materials with occupational exposure limits established by the region specific regulatory bodies.

Personal Protective Equipment

Eye/face Protection Wear appropriate protective eyeglasses or chemical safety goggles as described by OSHA's eye and face protection regulations in 29 CFR 1910.133 or European Standard EN166.

Skin and body protection No special protective equipment required.

Respiratory Protection No special protective equipment required.

9. PHYSICAL AND CHEMICAL PROPERTIES

Physical State	Liquid
Appearance	Clear, Colorless
Odor	Odorless
Odor Threshold	No information available
pH	No information available 7
Vapor Pressure	17.5 mmHg @ 20 °C
Vapor Density	No information available
Viscosity	No information available
Boiling Point/Range	100 °C / 212 °F
Melting Point/Range	0 °C / 32 °F
Decomposition Temperature	No information available
Flash Point	Not applicable
Evaporation Rate	No information available
Specific Gravity	1.000
Solubility	miscible
log Pow	No data available
Molecular Weight	18.02
Molecular Formula	H2O

10. STABILITY AND REACTIVITY

Stability Stable under normal conditions.

Conditions to Avoid Incompatible products. Excess heat. Avoid dust formation.

Incompatible Materials None known

Hazardous Decomposition Products	None
Hazardous Polymerization	Hazardous polymerization does not occur.
Hazardous Reactions	None under normal processing.

11. TOXICOLOGICAL INFORMATION

Acute Toxicity

Product Information	No acute toxicity information is available for this product
Component Information	
Irritation	No information available
Toxicologically Synergistic Products	No information available

Chronic Toxicity

Carcinogenicity	There are no known carcinogenic chemicals in this product.
Sensitization	No information available
Mutagenic Effects	No information available
Reproductive Effects	No information available.
Developmental Effects	No information available.
Teratogenicity	No information available.
Other Adverse Effects	No information available.
Endocrine Disruptor Information	No information available

12. ECOLOGICAL INFORMATION

Ecotoxicity
Contains no substances known to be hazardous to the environment or that are not degradable in waste water treatment plants.

Persistence and Degradability	No information available.
Bioaccumulation/ Accumulation	No information available.
Mobility	No information available.

13. DISPOSAL CONSIDERATIONS

Waste Disposal Methods	Chemical waste generators must determine whether a discarded chemical is classified as a hazardous waste. Chemical waste generators must also consult local, regional, and national hazardous waste regulations to ensure complete and accurate classification.

14. TRANSPORT INFORMATION

DOT	Not regulated

TDG Not regulated

IATA Not regulated

IMDG/IMO Not regulated

15. REGULATORY INFORMATION

All of the components in the product are on the following Inventory lists: Australia X = listed China Canada Europe TSCA Korea Philippines

International Inventories

Component	TSCA	DSL	NDSL	EINECS	ELINCS	NLP	PICCS	ENCS	AICS	IECSC	KECL
Water	X	X	-	231-791-2	-		X	-	X	X	X

Legend:

X - Listed

E - Indicates a substance that is the subject of a Section 5(e) Consent order under TSCA.

F - Indicates a substance that is the subject of a Section 5(f) Rule under TSCA.

N - Indicates a polymeric substance containing no free-radical initiator in its inventory name but is considered to cover the designated polymer made with any free-radical initiator regardless of the amount used.

P - Indicates a commenced PMN substance

R - Indicates a substance that is the subject of a Section 6 risk management rule under TSCA.

S - Indicates a substance that is identified in a proposed or final Significant New Use Rule

T - Indicates a substance that is the subject of a Section 4 test rule under TSCA.

XU - Indicates a substance exempt from reporting under the Inventory Update Rule, i.e. Partial Updating of the TSCA Inventory Data Base Production and Site Reports (40 CFR 710(B).

Y1 - Indicates an exempt polymer that has a number-average molecular weight of 1,000 or greater.

Y2 - Indicates an exempt polymer that is a polyester and is made only from reactants included in a specified list of low concern reactants that comprises one of the eligibility criteria for the exemption rule.

U.S. Federal Regulations

TSCA 12(b) Not applicable

SARA 313
Not applicable

SARA 311/312 Hazardous Categorization

Acute Health Hazard	No
Chronic Health Hazard	No
Fire Hazard	No
Sudden Release of Pressure Hazard	No
Reactive Hazard	No

Clean Water Act
Not applicable

Clean Air Act
Not applicable

OSHA Occupational Safety and Health Administration
Not applicable

CERCLA
Not applicable

California Proposition 65
This product does not contain any Proposition 65 chemicals

State Right-to-Know
Not applicable

U.S. Department of Transportation

Reportable Quantity (RQ):	N
DOT Marine Pollutant	N
DOT Severe Marine Pollutant	N

U.S. Department of Homeland Security
This product does not contain any DHS chemicals.

Other International Regulations

Mexico - Grade No information available

Canada

This product has been classified in accordance with the hazard criteria of the Controlled Products Regulations (CPR) and the MSDS contains all the information required by the CPR

WHMIS Hazard Class
Non-controlled

16. OTHER INFORMATION

Prepared By Regulatory Affairs
Thermo Fisher Scientific
Email: EMSDS.RA@thermofisher.com

Creation Date 26-Jan-2010

Print Date 28-Dec-2011

Revision Summary
"****", and red text indicates revision

Disclaimer
The information provided on this Safety Data Sheet is correct to the best of our knowledge, information and belief at the date of its publication. The information given is designed only as a guide for safe handling, use, processing, storage, transportation, disposal and release and is not to be considered as a warranty or quality specification. The information relates only to the specific material designated and may not be valid for such material used in combination with any other material or in any process, unless specified in the text.

End of MSDS

Fisher Scientific

Part of Thermo Fisher Scientific

Material Safety Data Sheet

Creation Date 05-May-2014 **Revision Date** 23-Oct-2014 **Revision Number** 1

1. PRODUCT AND COMPANY IDENTIFICATION

Product Name **Methanol**

Cat No. : **A452-1; A452-4; A452-4LC; A452N1-19; A452N2-19; A452POP-50; A452POP-200; A452RS-19; A452RS-28; A452RS-50; A452RS-115; A452RS-200; A452SK-1; A452SK-4; A452SS-19; A452SS-28; A452SS-50; A452SS-200**

Synonyms Methyl alcohol

Recommended Use Laboratory chemicals

Company
Fisher Scientific
One Reagent Lane
Fair Lawn, NJ 07410
Tel: (201) 796-7100

Emergency Telephone Number
CHEMTREC®, Inside the USA: 800-424-9300
CHEMTREC®, Outside the USA: 001-703-527-3887

2. HAZARDS IDENTIFICATION

DANGER

Emergency Overview

Flammable liquid and vapor. Poison, may be fatal or cause blindness if swallowed. Cannot be made non-poisonous. Vapor harmful. Toxic: danger of very serious irreversible effects through inhalation, in contact with skin and if swallowed. Irritating to eyes and skin. WARNING! This product contains a chemical known in the State of California to cause birth defects or other reproductive harm.

Appearance Colorless **Physical State** Liquid **Odor** Alcohol-like

Target Organs Gastrointestinal tract (GI), Central nervous system (CNS), Eyes, Respiratory system, Skin, Optic nerve, Liver, Kidney, spleen, Blood

Potential Health Effects

Acute Effects
Principle Routes of Exposure

Eyes Irritating to eyes.
Skin Toxic in contact with skin. Irritating to skin.
Inhalation Toxic by inhalation. Vapor harmful. May cause irritation of respiratory tract.

Ingestion Poison, may be fatal or cause blindness if swallowed. Cannot be made non-poisonous. Ingestion may cause gastrointestinal irritation, nausea, vomiting and diarrhea.

Chronic Effects
Toxic: danger of very serious irreversible effects through inhalation, in contact with skin and if swallowed: Experiments have shown reproductive toxicity effects on laboratory animals: May cause adverse liver effects: May cause adverse kidney effects: Component substance is listed on California Proposition 65 as a developmental hazard

Aggravated Medical Conditions Central nervous system disorders. Gastrointestinal tract. Preexisting eye disorders. Skin disorders. Kidney disorders. Liver disorders.

3. COMPOSITION/INFORMATION ON INGREDIENTS

Component	CAS-No	Weight %
Methyl alcohol	67-56-1	100

4. FIRST AID MEASURES

Eye Contact Rinse immediately with plenty of water, also under the eyelids, for at least 15 minutes. Immediate medical attention is required.

Skin Contact Wash off immediately with plenty of water for at least 15 minutes. Immediate medical attention is required.

Inhalation Move to fresh air. If breathing is difficult, give oxygen. Do not use mouth-to-mouth resuscitation if victim ingested or inhaled the substance; induce artificial respiration with a respiratory medical device. Immediate medical attention is required.

Ingestion Do not induce vomiting. Call a physician or Poison Control Center immediately.

Notes to Physician Treat symptomatically

5. FIRE-FIGHTING MEASURES

Flash Point 12 °C / 53.6 °F

Method - No information available

Autoignition Temperature 455 °C / 851 °F

Explosion Limits
Upper 31.00 vol %
Lower 6.0 vol %

Suitable Extinguishing Media CO_2, dry chemical, dry sand, alcohol-resistant foam. Use water spray to cool unopened containers. Cool closed containers exposed to fire with water spray.

Unsuitable Extinguishing Media Water may be ineffective

Hazardous Combustion Products No information available.

Sensitivity to Mechanical Impact No information available
Sensitivity to Static Discharge No information available

Specific Hazards Arising from the Chemical
Flammable. Risk of ignition. Vapors may form explosive mixtures with air. Vapors may travel to source of ignition and flash back. Containers may explode when heated. Vapors may form explosive mixtures with air.

Protective Equipment and Precautions for Firefighters
As in any fire, wear self-contained breathing apparatus pressure-demand, MSHA/NIOSH (approved or equivalent) and full protective gear. Thermal decomposition can lead to release of irritating gases and vapors.

NFPA	Health 1	Flammability 3	Instability 0	Physical hazards N/A

6. ACCIDENTAL RELEASE MEASURES

Personal Precautions	Remove all sources of ignition. Evacuate personnel to safe areas. Keep people away from and upwind of spill/leak. Use personal protective equipment. Ensure adequate ventilation. Take precautionary measures against static discharges.
Environmental Precautions	Should not be released into the environment.
Methods for Containment and Clean Up	Remove all sources of ignition. Soak up with inert absorbent material. Take precautionary measures against static discharges. Keep in suitable, closed containers for disposal. Use spark-proof tools and explosion-proof equipment.

7. HANDLING AND STORAGE

Handling	Wear personal protective equipment. Ensure adequate ventilation. Keep away from open flames, hot surfaces and sources of ignition. Take precautionary measures against static discharges. Do not breathe vapors or spray mist. Do not get in eyes, on skin, or on clothing. Use only non-sparking tools. Use explosion-proof equipment.
Storage	Keep containers tightly closed in a dry, cool and well-ventilated place. Keep away from open flames, hot surfaces and sources of ignition. Flammables area.

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Engineering Measures Use only under a chemical fume hood. Use explosion-proof electrical/ventilating/lighting/equipment. Ensure that eyewash stations and safety showers are close to the workstation location.

Exposure Guidelines

Component	ACGIH TLV	OSHA PEL	NIOSH IDLH
Methyl alcohol	TWA: 200 ppm STEL: 250 ppm Skin	(Vacated) TWA: 200 ppm (Vacated) TWA: 260 mg/m³ (Vacated) STEL: 250 ppm (Vacated) STEL: 325 mg/m³ Skin TWA: 200 ppm TWA: 260 mg/m³	IDLH: 6000 ppm TWA: 200 ppm TWA: 260 mg/m³ STEL: 250 ppm STEL: 325 mg/m³

Component	Quebec	Mexico OEL (TWA)	Ontario TWAEV
Methyl alcohol	TWA: 200 ppm TWA: 262 mg/m³ STEL: 250 ppm STEL: 328 mg/m³ Skin	TWA: 200 ppm TWA: 260 mg/m³ STEL: 250 ppm STEL: 310 mg/m³	TWA: 200 ppm STEL: 250 ppm Skin

Legend

NIOSH IDLH: The National Institute for Occupational Safety and Health Immediately Dangerous to Life or Health

Personal Protective Equipment

Eye/face Protection Wear appropriate protective eyeglasses or chemical safety goggles as described by OSHA's eye and face protection regulations in 29 CFR 1910.133 or European Standard EN166.

Skin and body protection Wear appropriate protective gloves and clothing to prevent skin exposure.

Respiratory Protection Follow the OSHA respirator regulations found in 29 CFR 1910.134 or European Standard EN 149. Use a NIOSH/MSHA or European Standard EN 149 approved respirator if exposure limits are exceeded or if irritation or other symptoms are experienced.

9. PHYSICAL AND CHEMICAL PROPERTIES

Physical State	Liquid
Appearance	Colorless
Odor	Alcohol-like
Odor Threshold	No information available
pH	No information available
Vapor Pressure	128 hPa @ 20 °C
Vapor Density	1.11
Viscosity	0.55 cP at 20 °C
Boiling Point/Range	64.7 °C / 148.5 °F @ 760 mmHg
Melting Point/Range	-98 °C / -144.4 °F
Decomposition Temperature	No information available
Flash Point	12 °C / 53.6 °F
Evaporation Rate	5.2 (ether = 1)
Specific Gravity	0.791
Solubility	Miscible with water
log Pow	No data available
Molecular Weight	32.04
Molecular Formula	C H4 O

10. STABILITY AND REACTIVITY

Stability Stable under normal conditions.

Conditions to Avoid Incompatible products. Heat, flames and sparks. Keep away from open flames, hot surfaces and sources of ignition.

Incompatible Materials Strong oxidizing agents, Strong acids, Acid anhydrides, Acid chlorides, Strong bases, Metals, Peroxides

Hazardous Decomposition Products Carbon monoxide (CO), Formaldehyde

Hazardous Polymerization Hazardous polymerization does not occur.

Hazardous Reactions None under normal processing.

11. TOXICOLOGICAL INFORMATION

Acute Toxicity

Product Information

Component Information

Component	LD50 Oral	LD50 Dermal	LC50 Inhalation
Methyl alcohol	6200 mg/kg (Rat)	Not listed	22500 ppm (Rat) 8 h

Irritation Irritating to eyes and skin

Toxicologically Synergistic Products Carbon tetrachloride

Chronic Toxicity

Carcinogenicity There are no known carcinogenic chemicals in this product.

Sensitization No information available

Mutagenic Effects Mutagenic effects have occurred in experimental animals.

Reproductive Effects Experiments have shown reproductive toxicity effects on laboratory animals.

Developmental Effects Developmental effects have occurred in experimental animals. Component substance is listed on California Proposition 65 as a developmental hazard.

Teratogenicity Teratogenic effects have occurred in experimental animals.

Other Adverse Effects The toxicological properties have not been fully investigated.

Endocrine Disruptor Information No information available

12. ECOLOGICAL INFORMATION

Ecotoxicity

Component	Freshwater Algae	Freshwater Fish	Microtox	Water Flea

Methyl alcohol	Not listed	Pimephales promelas: LC50 > 10000 mg/L 96h	EC50 = 39000 mg/L 25 min EC50 = 40000 mg/L 15 min EC50 = 43000 mg/L 5 min	EC50 > 10000 mg/L 24h

Persistence and Degradability Readily biodegradable.

Bioaccumulation/ Accumulation No information available.

Mobility Will likely be mobile in the environment due to its volatility.

Component	log Pow
Methyl alcohol	-0.74

13. DISPOSAL CONSIDERATIONS

Waste Disposal Methods Should not be released into the environment.

Component	RCRA - U Series Wastes	RCRA - P Series Wastes
Methyl alcohol - 67-56-1	U154	-

14. TRANSPORT INFORMATION

DOT

UN-No	UN1230
Proper Shipping Name	METHANOL
Hazard Class	3
Packing Group	II

TDG

UN-No	UN1230
Proper Shipping Name	METHANOL
Hazard Class	3
Subsidiary Hazard Class	6.1
Packing Group	II

IATA

UN-No	UN1230
Proper Shipping Name	METHANOL
Hazard Class	3
Subsidiary Hazard Class	6.1
Packing Group	II

IMDG/IMO

UN-No	UN1230
Proper Shipping Name	METHANOL
Hazard Class	3
Subsidiary Hazard Class	6.1
Packing Group	II

15. REGULATORY INFORMATION

International Inventories

Component	TSCA	DSL	NDSL	EINECS	ELINCS	NLP	PICCS	ENCS	AICS	IECSC	KECL
Methyl alcohol	X	X	-	200-659-6	-		X	X	X	X	X

Legend:

X - Listed

E - Indicates a substance that is the subject of a Section 5(e) Consent order under TSCA.

F - Indicates a substance that is the subject of a Section 5(f) Rule under TSCA.

N - Indicates a polymeric substance containing no free-radical initiator in its inventory name but is considered to cover the designated polymer made with any free-radical initiator regardless of the amount used.

P - Indicates a commenced PMN substance

R - Indicates a substance that is the subject of a Section 6 risk management rule under TSCA.

S - Indicates a substance that is identified in a proposed or final Significant New Use Rule

T - Indicates a substance that is the subject of a Section 4 test rule under TSCA.

XU - Indicates a substance exempt from reporting under the Inventory Update Rule, i.e. Partial Updating of the TSCA Inventory Data Base Production and Site Reports (40 CFR 710(B).

Y1 - Indicates an exempt polymer that has a number-average molecular weight of 1,000 or greater.

Y2 - Indicates an exempt polymer that is a polyester and is made only from reactants included in a specified list of low concern reactants that comprises one of the eligibility criteria for the exemption rule.

U.S. Federal Regulations

TSCA 12(b) Not applicable

SARA 313

Component	CAS-No	Weight %	SARA 313 - Threshold Values %
Methyl alcohol	67-56-1	100	1.0

SARA 311/312 Hazardous Categorization

Acute Health Hazard	Yes
Chronic Health Hazard	Yes
Fire Hazard	Yes
Sudden Release of Pressure Hazard	No
Reactive Hazard	No

Clean Water Act
Not applicable

Clean Air Act

Component	HAPS Data	Class 1 Ozone Depletors	Class 2 Ozone Depletors
Methyl alcohol 67-56-1 (100)	X		-

OSHA Occupational Safety and Health Administration
Not applicable

CERCLA
This material, as supplied, contains one or more substances regulated as a hazardous substance under the Comprehensive Environmental Response Compensation and Liability Act (CERCLA) (40 CFR 302)

Component	Hazardous Substances RQs	CERCLA EHS RQs
Methyl alcohol	5000 lb	-

California Proposition 65

This product contains the following Proposition 65 chemicals:

Component	CAS-No	California Prop. 65	Prop 65 NSRL	Category
Methyl alcohol	67-56-1	Developmental	-	Developmental

State Right-to-Know

Component	Massachusetts	New Jersey	Pennsylvania	Illinois	Rhode Island
Methyl alcohol	X	X	X	X	X

U.S. Department of Transportation

Reportable Quantity (RQ): Y
DOT Marine Pollutant N
DOT Severe Marine Pollutant N

U.S. Department of Homeland Security

This product does not contain any DHS chemicals.

Other International Regulations

Mexico - Grade Serious risk, Grade 3

Canada

This product has been classified in accordance with the hazard criteria of the Controlled Products Regulations (CPR) and the MSDS contains all the information required by the CPR

WHMIS Hazard Class
B2 Flammable liquid
D1A Very toxic materials
D2A Very toxic materials

16. OTHER INFORMATION

Prepared By Regulatory Affairs
Thermo Fisher Scientific
Email: EMSDS.RA@thermofisher.com

Creation Date 05-May-2014

Print Date 23-Oct-2014

Revision Summary
Update to Format (M)SDS sections updated 4 8 11 12 13 15 16

Disclaimer

The information provided on this Safety Data Sheet is correct to the best of our knowledge, information and belief at the date of its publication. The information given is designed only as a guide for safe handling, use, processing, storage, transportation, disposal and release and is not to be considered as a warranty or quality specification. The information relates only to the specific material designated and may not be valid for such material used in combination with any other material or in any process, unless specified in the text.

End of MSDS

Fisher Scientific

Part of Thermo Fisher Scientific

Material Safety Data Sheet

Creation Date 05-May-2009 **Revision Date** 13-Feb-2013 **Revision Number** 3

1. PRODUCT AND COMPANY IDENTIFICATION

Product Name **Acetic acid**

Cat No. **A35-500; A38-212; A38-450LB; A38-500; A38-500LC; A38C-212; A38P-20; A38P-500; A38S-212; A38S-500; A38SI-212; A465-1; A465-250; A465-500; A490-212; A491-212; BP1185-500; BP1185-500LC; BP2400-500; BP2401-212; BP2401-500; BP2401C-212; BP2401P-20; BP2401S-212; BP2401S-500; BP2401SI-212; S700481**

Synonyms Glacial acetic acid; Methanecarboxylic acid; Ethanoic acid; Vinegar acid (HPLC/Certified ACS/OPTIMA//USP/FCC/EP/BP/Trace Metal Grade/Aldehyde-Free/Sequencing)

Recommended Use Laboratory chemicals

Company
Fisher Scientific
One Reagent Lane
Fair Lawn, NJ 07410
Tel: (201) 796-7100

Emergency Telephone Number
CHEMTREC®, Inside the USA: 800-424-9300
CHEMTREC®, Outside the USA: 001-703-527-3887

2. HAZARDS IDENTIFICATION

DANGER!

Emergency Overview

Flammable liquid and vapor. Causes severe burns by all exposure routes.

Appearance Colorless **Physical State** Liquid **Odor** vinegar-like

Target Organs Eyes, Respiratory system, Skin, Teeth, Gastrointestinal tract (GI), Liver, Kidney, Blood

Potential Health Effects

Acute Effects
Principle Routes of Exposure

Eyes	Causes severe burns. May cause blindness or permanent eye damage.
Skin	Causes severe burns. May be harmful in contact with skin.
Inhalation	Causes severe burns. May be harmful if inhaled.
Ingestion	Causes severe burns. May be harmful if swallowed.

Chronic Effects Experiments have shown reproductive toxicity effects on laboratory animals. May cause adverse liver effects. May cause adverse kidney effects. Chronic exposure to corrosive fumes/gases may cause erosion of the teeth followed by jaw necrosis. Bronchial irritation with chronic cough and frequent attacks of pneumonia are common. Gastrointestinal disturbances may also be seen.

See Section 11 for additional Toxicological information.

Aggravated Medical Conditions Preexisting eye disorders. Skin disorders. Gastrointestinal tract.

3. COMPOSITION/INFORMATION ON INGREDIENTS

Haz/Non-haz

Component	CAS-No	Weight %
Acetic acid	64-19-7	>95

4. FIRST AID MEASURES

Eye Contact Rinse immediately with plenty of water, also under the eyelids, for at least 15 minutes. Immediate medical attention is required.

Skin Contact Wash off immediately with plenty of water for at least 15 minutes. Immediate medical attention is required.

Inhalation Move to fresh air. If breathing is difficult, give oxygen. Do not use mouth-to-mouth resuscitation if victim ingested or inhaled the substance; induce artificial respiration with a respiratory medical device. Immediate medical attention is required.

Ingestion Do not induce vomiting. Call a physician or Poison Control Center immediately.

Notes to Physician Treat symptomatically.

5. FIRE-FIGHTING MEASURES

Flash Point 40°C / 104°F

Method - No information available.

Autoignition Temperature 427°C / 800.6°F

Explosion Limits
Upper 19.9 vol %
Lower 4.0 vol %

Suitable Extinguishing Media Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

Unsuitable Extinguishing Media No information available.

Hazardous Combustion Products No information available.

Sensitivity to mechanical impact No information available.
Sensitivity to static discharge No information available.

Specific Hazards Arising from the Chemical
Flammable. Risk of ignition. Vapors may form explosive mixtures with air. Vapors may travel to source of ignition and flash back. Containers may explode when heated. Causes severe burns by all exposure routes. Contact with metals may evolve flammable hydrogen gas.

Protective Equipment and Precautions for Firefighters
As in any fire, wear self-contained breathing apparatus pressure-demand, MSHA/NIOSH (approved or equivalent) and full protective gear. Thermal decomposition can lead to release of irritating gases and vapors.

NFPA **Health** 3 **Flammability** 2 **Instability** 0 **Physical hazards** N/A

6. ACCIDENTAL RELEASE MEASURES

Personal Precautions Remove all sources of ignition. Use personal protective equipment. Evacuate personnel to safe areas. Keep people away from and upwind of spill/leak. Take precautionary measures against static discharges. Do not get in eyes, on skin, or on clothing.

Environmental Precautions Should not be released into the environment.

Methods for Containment and Clean Up Remove all sources of ignition. Soak up with inert absorbent material. Use spark-proof tools and explosion-proof equipment. Keep in suitable, closed containers for disposal..

7. HANDLING AND STORAGE

Handling Use only under a chemical fume hood. Use explosion-proof equipment. Keep away from open flames, hot surfaces and sources of ignition. Do not breathe vapors or spray mist. Do not get in eyes, on skin, or on clothing. Take precautionary measures against static discharges.

Storage Keep containers tightly closed in a dry, cool and well-ventilated place. Keep away from open flames, hot surfaces and sources of ignition. Flammables area.

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Engineering Measures Use only under a chemical fume hood. Use explosion-proof electrical/ventilating/lighting/equipment. Ensure that eyewash stations and safety showers are close to the workstation location.

Exposure Guidelines

Component	ACGIH TLV	OSHA PEL	NIOSH IDLH
Acetic acid	TWA: 10 ppm STEL: 15 ppm	(Vacated) TWA: 10 ppm (Vacated) TWA: 25 mg/m^3 TWA: 10 ppm TWA: 25 mg/m^3	IDLH: 50 ppm TWA: 10 ppm TWA: 25 mg/m^3 STEL: 15 ppm STEL: 37 mg/m^3

Component	Quebec	Mexico OEL (TWA)	Ontario TWAEV
Acetic acid	TWA: 10 ppm TWA: 25 mg/m^3 STEL: 15 ppm STEL: 37 mg/m^3	TWA: 10 ppm TWA: 25 mg/m^3 STEL: 15 ppm STEL: 37 mg/m^3	TWA: 10 ppm STEL: 15 ppm

NIOSH IDLH: *Immediately Dangerous to Life or Health*

Personal Protective Equipment

Eye/face Protection Wear appropriate protective eyeglasses or chemical safety goggles as described by OSHA's eye and face protection regulations in 29 CFR 1910.133 or European Standard EN166.

Skin and body protection Wear appropriate protective gloves and clothing to prevent skin exposure.

Respiratory Protection Follow the OSHA respirator regulations found in 29 CFR 1910.134 or European Standard EN 149. Use a NIOSH/MSHA or European Standard EN 149 approved respirator if exposure limits are exceeded or if irritation or other symptoms are experienced.

9. PHYSICAL AND CHEMICAL PROPERTIES

Physical State	Liquid
Appearance	Colorless
Odor	vinegar-like
Odor Threshold	No information available.
pH	< 2.5 10 g/L aq.sol.
Vapor Pressure	1.52 kPa @ 20 °C
Vapor Density	2.10 (Air = 1.0)
Viscosity	1.53 mPa.s @ 25 °C
Boiling Point/Range	117 - 118°C / 242.6 - 244.4°F
Melting Point/Range	16 - 16.5°C / 60.8 - 61.7°F
Decomposition temperature	No information available.
Flash Point	40°C / 104°F
Evaporation Rate	0.97 (Butyl Acetate = 1.0)
Specific Gravity	1.048
Solubility	Soluble in water
log Pow	No data available
Molecular Weight	60.05
Molecular Formula	C2 H4 O2

10. STABILITY AND REACTIVITY

Stability	Hygroscopic.
Conditions to Avoid	Incompatible products. Heat, flames and sparks. Exposure to moist air or water.
Incompatible Materials	Strong oxidizing agents, Strong bases, Metals
Hazardous Decomposition Products	Carbon monoxide (CO), Carbon dioxide (CO_2)
Hazardous Polymerization	Hazardous polymerization does not occur.
Hazardous Reactions .	None under normal processing.

11. TOXICOLOGICAL INFORMATION

Acute Toxicity

Product Information

Component Information

Component	LD50 Oral	LD50 Dermal	LC50 Inhalation
Acetic acid	3310 mg/kg (Rat)	1060 mg/kg (Rabbit)	11.4 mg/L (Rat) 4 h

Irritation	Causes severe burns by all exposure routes
Toxicologically Synergistic Products	No information available.

Chronic Toxicity

Carcinogenicity	There are no known carcinogenic chemicals in this product

Sensitization	No information available.
Mutagenic Effects	Not mutagenic in AMES Test
Reproductive Effects	Experiments have shown reproductive toxicity effects on laboratory animals.
Developmental Effects	No information available.
Teratogenicity	No information available.
Other Adverse Effects	See actual entry in RTECS for complete information.
Endocrine Disruptor Information	No information available

12. ECOLOGICAL INFORMATION

Ecotoxicity

.

Component	Freshwater Algae	Freshwater Fish	Microtox	Water Flea
Acetic acid	Not listed	Pimephales promelas: LC50 = 88 mg/L/96h Lepomis macrochirus: LC50 = 75 mg/L/96h	Photobacterium phosphoreum: EC50 = 8.8 mg/L/15 min Photobacterium phosphoreum: EC50 = 8.8 mg/L/25 min Photobacterium phosphoreum: EC50 = 8.8 mg/L/5 min	EC50 = 95 mg/L/24h

Persistence and Degradability	Expected to be biodegradable.
Bioaccumulation/ Accumulation	No information available
Mobility	.

Component	log Pow
Acetic acid	-0.2

13. DISPOSAL CONSIDERATIONS

Waste Disposal Methods Chemical waste generators must determine whether a discarded chemical is classified as a hazardous waste. Chemical waste generators must also consult local, regional, and national hazardous waste regulations to ensure complete and accurate classification.

14. TRANSPORT INFORMATION

DOT

UN-No	UN2789
Proper Shipping Name	Acetic acid, glacial
Hazard Class	8
Subsidiary Hazard Class	3
Packing Group	II

TDG

14. TRANSPORT INFORMATION

UN-No	UN2789
Proper Shipping Name	ACETIC ACID, GLACIAL
Hazard Class	8
Subsidiary Hazard Class	3
Packing Group	II

IATA

UN-No	UN2789
Proper Shipping Name	ACETIC ACID, GLACIAL
Hazard Class	8
Subsidiary Hazard Class	3
Packing Group	II

IMDG/IMO

UN-No	UN2789
Proper Shipping Name	ACETIC ACID, GLACIAL
Hazard Class	8
Subsidiary Hazard Class	3
Packing Group	II

15. REGULATORY INFORMATION

International Inventories

Component	TSCA	DSL	NDSL	EINECS	ELINCS	NLP	PICCS	ENCS	AICS	CHINA	KECL
Acetic acid	X	X	-	200-580-7	-		X	X	X	X	X

Legend:
X - Listed
E - Indicates a substance that is the subject of a Section 5(e) Consent order under TSCA.
F - Indicates a substance that is the subject of a Section 5(f) Rule under TSCA.
N - Indicates a polymeric substance containing no free-radical initiator in its inventory name but is considered to cover the designated polymer made with any free-radical initiator regardless of the amount used.
P - Indicates a commenced PMN substance
R - Indicates a substance that is the subject of a Section 6 risk management rule under TSCA.
S - Indicates a substance that is identified in a proposed or final Significant New Use Rule
T - Indicates a substance that is the subject of a Section 4 test rule under TSCA.
XU - Indicates a substance exempt from reporting under the Inventory Update Rule, i.e. Partial Updating of the TSCA Inventory Data Base Production and Site Reports (40 CFR 710(B).
Y1 - Indicates an exempt polymer that has a number-average molecular weight of 1,000 or greater.
Y2 - Indicates an exempt polymer that is a polyester and is made only from reactants included in a specified list of low concern reactants that comprises one of the eligibility criteria for the exemption rule.

U.S. Federal Regulations

TSCA 12(b) Not applicable

SARA 313
Not applicable

SARA 311/312 Hazardous Categorization

Acute Health Hazard	Yes
Chronic Health Hazard	No

Fire Hazard	Yes
Sudden Release of Pressure Hazard	No
Reactive Hazard	No

Clean Water Act

Component	CWA - Hazardous Substances	CWA - Reportable Quantities	CWA - Toxic Pollutants	CWA - Priority Pollutants
Acetic acid	X	5000 lb	-	-

Clean Air Act
Not applicable

OSHA
Not applicable

CERCLA
This material, as supplied, contains one or more substances regulated as a hazardous substance under the Comprehensive Environmental Response Compensation and Liability Act (CERCLA) (40 CFR 302)

Component	Hazardous Substances RQs	CERCLA EHS RQs
Acetic acid	5000 lb	-

California Proposition 65
This product does not contain any Proposition 65 chemicals.

State Right-to-Know

Component	Massachusetts	New Jersey	Pennsylvania	Illinois	Rhode Island
Acetic acid	X	X	X	-	X

U.S. Department of Transportation

Reportable Quantity (RQ):	Y
DOT Marine Pollutant	N
DOT Severe Marine Pollutant	N

U.S. Department of Homeland Security
This product does not contain any DHS chemicals.

Other International Regulations

Mexico - Grade Moderate risk, Grade 2

Canada

This product has been classified in accordance with the hazard criteria of the Controlled Products Regulations (CPR) and the MSDS contains all the information required by the CPR.

WHMIS Hazard Class
B3 Combustible liquid
E Corrosive material

16. OTHER INFORMATION

Prepared By	Regulatory Affairs Thermo Fisher Scientific Email: EMSDS.RA@thermofisher.com
Creation Date	05-May-2009
Print Date	13-Feb-2013
Revision Summary	"****", and red text indicates revision

Disclaimer
The information provided on this Safety Data Sheet is correct to the best of our knowledge, information and belief at the date of its publication. The information given is designed only as a guide for safe handling, use, processing, storage, transportation, disposal and release and is not to be considered as a warranty or quality specification. The information relates only to the specific material designated and may not be valid for such material used in combination with any other material or in any process, unless specified in the text.

End of MSDS

SAFETY DATA SHEET

Version 3.11
Revision Date 06/19/2014
Print Date 03/30/2015

1. PRODUCT AND COMPANY IDENTIFICATION

1.1 Product identifiers

Product name : Capsaicin

Product Number : 21750
Brand : Sigma

CAS-No. : 404-86-4

1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses : Laboratory chemicals, Manufacture of substances

1.3 Details of the supplier of the safety data sheet

Company : Sigma-Aldrich
3050 Spruce Street
SAINT LOUIS MO 63103
USA

Telephone : +1 800-325-5832
Fax : +1 800-325-5052

1.4 Emergency telephone number

Emergency Phone # : (314) 776-6555

2. HAZARDS IDENTIFICATION

2.1 Classification of the substance or mixture

GHS Classification in accordance with 29 CFR 1910 (OSHA HCS)
Acute toxicity, Oral (Category 3), H301
Skin irritation (Category 2), H315
Eye irritation (Category 2A), H319
Respiratory sensitisation (Category 1), H334
Skin sensitisation (Category 1), H317
Specific target organ toxicity - single exposure (Category 3), Respiratory system, H335

For the full text of the H-Statements mentioned in this Section, see Section 16.

2.2 GHS Label elements, including precautionary statements

Pictogram

Signal word Danger

Hazard statement(s)
H301 Toxic if swallowed.
H315 Causes skin irritation.
H317 May cause an allergic skin reaction.
H319 Causes serious eye irritation.
H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H335 May cause respiratory irritation.

Precautionary statement(s)
P261 Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray.
P264 Wash skin thoroughly after handling.

P270	Do not eat, drink or smoke when using this product.
P271	Use only outdoors or in a well-ventilated area.
P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves/ eye protection/ face protection.
P285	In case of inadequate ventilation wear respiratory protection.
P301 + P310	IF SWALLOWED: Immediately call a POISON CENTER or doctor/ physician.
P302 + P352	IF ON SKIN: Wash with plenty of soap and water.
P304 + P340	IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.
P305 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P321	Specific treatment (see supplemental first aid instructions on this label).
P330	Rinse mouth.
P333 + P313	If skin irritation or rash occurs: Get medical advice/ attention.
P337 + P313	If eye irritation persists: Get medical advice/ attention.
P342 + P311	If experiencing respiratory symptoms: Call a POISON CENTER or doctor/ physician.
P362	Take off contaminated clothing and wash before reuse.
P403 + P233	Store in a well-ventilated place. Keep container tightly closed.
P405	Store locked up.
P501	Dispose of contents/ container to an approved waste disposal plant.

2.3 Hazards not otherwise classified (HNOC) or not covered by GHS

Lachrymator.
Lachrymator., Sternutator.

3. COMPOSITION/INFORMATION ON INGREDIENTS

3.1 Substances

Synonyms	:	8-Methyl-N-vanillyl-trans-6-nonenamide
Formula	:	$C_{18}H_{27}NO_3$
Molecular Weight	:	305.41 g/mol
CAS-No.	:	404-86-4
EC-No.	:	206-969-8

Hazardous components

Component	Classification	Concentration
Capsaicin		
	Acute Tox. 3; Skin Irrit. 2; Eye Irrit. 2A; Resp. Sens. 1; Skin Sens. 1; STOT SE 3; H301, H315, H317, H319, H334, H335	90 - 100 %

For the full text of the H-Statements mentioned in this Section, see Section 16.

4. FIRST AID MEASURES

4.1 Description of first aid measures

General advice
Consult a physician. Show this safety data sheet to the doctor in attendance.Move out of dangerous area.

If inhaled
If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.

In case of skin contact
Wash off with soap and plenty of water. Take victim immediately to hospital. Consult a physician.

In case of eye contact
Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician.

If swallowed
Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

4.2 Most important symptoms and effects, both acute and delayed
The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11

4.3 Indication of any immediate medical attention and special treatment needed
no data available

5. FIREFIGHTING MEASURES

5.1 Extinguishing media

Suitable extinguishing media
Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

5.2 Special hazards arising from the substance or mixture
Carbon oxides, nitrogen oxides (NOx)

5.3 Advice for firefighters
Wear self contained breathing apparatus for fire fighting if necessary.

5.4 Further information
no data available

6. ACCIDENTAL RELEASE MEASURES

6.1 Personal precautions, protective equipment and emergency procedures
Wear respiratory protection. Avoid dust formation. Avoid breathing vapours, mist or gas. Ensure adequate ventilation.
Evacuate personnel to safe areas. Avoid breathing dust.
For personal protection see section 8.

6.2 Environmental precautions
Prevent further leakage or spillage if safe to do so. Do not let product enter drains.

6.3 Methods and materials for containment and cleaning up
Pick up and arrange disposal without creating dust. Sweep up and shovel. Keep in suitable, closed containers for disposal.

6.4 Reference to other sections
For disposal see section 13.

7. HANDLING AND STORAGE

7.1 Precautions for safe handling
Avoid contact with skin and eyes. Avoid formation of dust and aerosols.
Provide appropriate exhaust ventilation at places where dust is formed.
For precautions see section 2.2.

7.2 Conditions for safe storage, including any incompatibilities
Keep container tightly closed in a dry and well-ventilated place.

Recommended storage temperature: 2 - 8 °C

7.3 Specific end use(s)
Apart from the uses mentioned in section 1.2 no other specific uses are stipulated

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

8.1 Control parameters

Components with workplace control parameters
Contains no substances with occupational exposure limit values.

8.2 Exposure controls

Appropriate engineering controls
Avoid contact with skin, eyes and clothing. Wash hands before breaks and immediately after handling the product.

Personal protective equipment

Eye/face protection
Face shield and safety glasses Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).

Skin protection
Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.

Full contact
Material: Nitrile rubber
Minimum layer thickness: 0.11 mm
Break through time: 480 min
Material tested:Dermatril® (KCL 740 / Aldrich Z677272, Size M)

Splash contact
Material: Nitrile rubber
Minimum layer thickness: 0.11 mm
Break through time: 480 min
Material tested:Dermatril® (KCL 740 / Aldrich Z677272, Size M)

data source: KCL GmbH, D-36124 Eichenzell, phone +49 (0)6659 87300, e-mail sales@kcl.de, test method: EN374
If used in solution, or mixed with other substances, and under conditions which differ from EN 374, contact the supplier of the CE approved gloves. This recommendation is advisory only and must be evaluated by an industrial hygienist and safety officer familiar with the specific situation of anticipated use by our customers. It should not be construed as offering an approval for any specific use scenario.

Body Protection
Complete suit protecting against chemicals, The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

Respiratory protection
Where risk assessment shows air-purifying respirators are appropriate use a full-face particle respirator type N99 (US) or type P2 (EN 143) respirator cartridges as a backup to engineering controls. If the respirator is the sole means of protection, use a full-face supplied air respirator. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

Control of environmental exposure
Prevent further leakage or spillage if safe to do so. Do not let product enter drains.

9. PHYSICAL AND CHEMICAL PROPERTIES

9.1 Information on basic physical and chemical properties

a)	Appearance	Form: crystalline Colour: white
b)	Odour	no data available
c)	Odour Threshold	no data available
d)	pH	no data available
e)	Melting point/freezing point	Melting point/range: 62 - 65 °C (144 - 149 °F) Melting point/range: 62 - 65 °C (144 - 149 °F)
f)	Initial boiling point and boiling range	no data available
g)	Flash point	113 °C (235 °F) - closed cup
h)	Evapouration rate	no data available
i)	Flammability (solid, gas)	no data available
j)	Upper/lower flammability or	no data available

	explosive limits	
k)	Vapour pressure	no data available
l)	Vapour density	no data available
m)	Relative density	no data available
n)	Water solubility	insoluble
o)	Partition coefficient: n-octanol/water	no data available
p)	Auto-ignition temperature	no data available
q)	Decomposition temperature	no data available
r)	Viscosity	no data available
s)	Explosive properties	no data available
t)	Oxidizing properties	no data available

9.2 Other safety information

Solubility in other solvents	Benzene - soluble Ether - soluble Chloroform - soluble

10. STABILITY AND REACTIVITY

10.1 Reactivity
no data available

10.2 Chemical stability
Stable under recommended storage conditions.

10.3 Possibility of hazardous reactions
no data available

10.4 Conditions to avoid
no data available

10.5 Incompatible materials
Strong oxidizing agents

10.6 Hazardous decomposition products
Other decomposition products - no data available
In the event of fire: see section 5

11. TOXICOLOGICAL INFORMATION

11.1 Information on toxicological effects

Acute toxicity
LD50 Oral - rat - male - 161.2 mg/kg

LD50 Oral - rat - female - 148.1 mg/kg

Inhalation: no data available

LD50 Dermal - mouse - > 512 mg/kg

no data available

Skin corrosion/irritation
no data available

Serious eye damage/eye irritation
Eyes - guinea pig
Result: Mild eye irritation

Respiratory or skin sensitisation
no data available

Germ cell mutagenicity
no data available

Carcinogenicity

IARC: No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.

ACGIH: No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by ACGIH.

NTP: No component of this product present at levels greater than or equal to 0.1% is identified as a known or anticipated carcinogen by NTP.

OSHA: No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by OSHA.

Reproductive toxicity
no data available

no data available

Specific target organ toxicity - single exposure
no data available

Specific target organ toxicity - repeated exposure
no data available

Aspiration hazard
no data available

Additional Information
RTECS: RA8530000

burning sensation, Cough, sneezing, wheezing, laryngitis, Shortness of breath, Headache, Nausea, Vomiting, To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

Liver - Irregularities - Based on Human Evidence
Liver - Irregularities - Based on Human Evidence

12. ECOLOGICAL INFORMATION

12.1 Toxicity
no data available

12.2 Persistence and degradability
no data available

12.3 Bioaccumulative potential
no data available

12.4 Mobility in soil
no data available

12.5 Results of PBT and vPvB assessment
PBT/vPvB assessment not available as chemical safety assessment not required/not conducted

12.6 Other adverse effects

no data available

13. DISPOSAL CONSIDERATIONS

13.1 Waste treatment methods

Product
Contact a licensed professional waste disposal service to dispose of this material. Dissolve or mix the material with a combustible solvent and burn in a chemical incinerator equipped with an afterburner and scrubber. Offer surplus and non-recyclable solutions to a licensed disposal company.

Contaminated packaging
Dispose of as unused product.

14. TRANSPORT INFORMATION

DOT (US)
UN number: 2811 Class: 6.1 Packing group: III
Proper shipping name: Toxic solids, organic, n.o.s. (Capsaicin)
Reportable Quantity (RQ):
Marine pollutant: No
Poison Inhalation Hazard: No

IMDG
UN number: 2811 Class: 6.1 Packing group: III EMS-No: F-A, S-A
Proper shipping name: TOXIC SOLID, ORGANIC, N.O.S. (Capsaicin)
Marine pollutant: No

IATA
UN number: 2811 Class: 6.1 Packing group: III
Proper shipping name: Toxic solid, organic, n.o.s. (Capsaicin)

15. REGULATORY INFORMATION

SARA 302 Components
SARA 302: No chemicals in this material are subject to the reporting requirements of SARA Title III, Section 302.

SARA 313 Components
SARA 313: This material does not contain any chemical components with known CAS numbers that exceed the threshold (De Minimis) reporting levels established by SARA Title III, Section 313.

SARA 311/312 Hazards
Acute Health Hazard, Chronic Health Hazard

Massachusetts Right To Know Components
No components are subject to the Massachusetts Right to Know Act.

Pennsylvania Right To Know Components

	CAS-No.	Revision Date
Capsaicin	404-86-4	

New Jersey Right To Know Components

	CAS-No.	Revision Date
Capsaicin	404-86-4	

California Prop. 65 Components
This product does not contain any chemicals known to State of California to cause cancer, birth defects, or any other reproductive harm.

16. OTHER INFORMATION

Full text of H-Statements referred to under sections 2 and 3.

Acute Tox.	Acute toxicity
Eye Irrit.	Eye irritation
H301	Toxic if swallowed.
H315	Causes skin irritation.
H317	May cause an allergic skin reaction.
H319	Causes serious eye irritation.
H334	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H335	May cause respiratory irritation.
Resp. Sens.	Respiratory sensitisation
Skin Irrit.	Skin irritation

HMIS Rating

Health hazard:	2
Chronic Health Hazard:	*
Flammability:	1
Physical Hazard	0

NFPA Rating

Health hazard:	2
Fire Hazard:	1
Reactivity Hazard:	0

Further information

Copyright 2014 Sigma-Aldrich Co. LLC. License granted to make unlimited paper copies for internal use only.
The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Corporation and its Affiliates shall not be held liable for any damage resulting from handling or from contact with the above product. See www.sigma-aldrich.com and/or the reverse side of invoice or packing slip for additional terms and conditions of sale.

Preparation Information

Sigma-Aldrich Corporation
Product Safety – Americas Region
1-800-521-8956

Version: 3.11 Revision Date: 06/19/2014 Print Date: 03/30/2015

SAFETY DATA SHEET

Version 5.3
Revision Date 08/27/2014
Print Date 03/30/2015

1. PRODUCT AND COMPANY IDENTIFICATION

1.1 Product identifiers

Product name : Dihydrocapsaicin

Product Number : 03813
Brand : Fluka

CAS-No. : 19408-84-5

1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses : Laboratory chemicals, Manufacture of substances

1.3 Details of the supplier of the safety data sheet

Company : Sigma-Aldrich
3050 Spruce Street
SAINT LOUIS MO 63103
USA

Telephone : +1 800-325-5832
Fax : +1 800-325-5052

1.4 Emergency telephone number

Emergency Phone # : (314) 776-6555

2. HAZARDS IDENTIFICATION

2.1 Classification of the substance or mixture

GHS Classification in accordance with 29 CFR 1910 (OSHA HCS)
Acute toxicity, Oral (Category 3), H301
Skin irritation (Category 2), H315
Eye irritation (Category 2A), H319
Specific target organ toxicity - single exposure (Category 3), Respiratory system, H335

For the full text of the H-Statements mentioned in this Section, see Section 16.

2.2 GHS Label elements, including precautionary statements

Pictogram

Signal word Danger

Hazard statement(s)
H301 Toxic if swallowed.
H315 Causes skin irritation.
H319 Causes serious eye irritation.
H335 May cause respiratory irritation.

Precautionary statement(s)
P261 Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray.
P264 Wash skin thoroughly after handling.
P270 Do not eat, drink or smoke when using this product.
P271 Use only outdoors or in a well-ventilated area.
P280 Wear protective gloves/ eye protection/ face protection.
P301 + P310 IF SWALLOWED: Immediately call a POISON CENTER or doctor/

	physician.
P302 + P352	IF ON SKIN: Wash with plenty of soap and water.
P304 + P340	IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.
P305 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P312	Call a POISON CENTER or doctor/ physician if you feel unwell.
P321	Specific treatment (see supplemental first aid instructions on this label).
P330	Rinse mouth.
P332 + P313	If skin irritation occurs: Get medical advice/ attention.
P337 + P313	If eye irritation persists: Get medical advice/ attention.
P362	Take off contaminated clothing and wash before reuse.
P403 + P233	Store in a well-ventilated place. Keep container tightly closed.
P405	Store locked up.
P501	Dispose of contents/ container to an approved waste disposal plant.

2.3 Hazards not otherwise classified (HNOC) or not covered by GHS - none

3. COMPOSITION/INFORMATION ON INGREDIENTS

3.1 Substances

Formula : $C_{18}H_{29}NO_3$
Molecular weight : 307.43 g/mol
CAS-No. : 19408-84-5

Hazardous components

Component	Classification	Concentration
8-Methyl-N-vanillylnonanamide		
	Acute Tox. 3; Skin Irrit. 2; Eye Irrit. 2A; STOT SE 3; H301, H315, H319, H335	-

For the full text of the H-Statements mentioned in this Section, see Section 16.

4. FIRST AID MEASURES

4.1 Description of first aid measures

General advice
Consult a physician. Show this safety data sheet to the doctor in attendance.Move out of dangerous area.

If inhaled
If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.

In case of skin contact
Wash off with soap and plenty of water. Take victim immediately to hospital. Consult a physician.

In case of eye contact
Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician.

If swallowed
Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

4.2 Most important symptoms and effects, both acute and delayed
The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11

4.3 Indication of any immediate medical attention and special treatment needed
No data available

5. FIREFIGHTING MEASURES

5.1 Extinguishing media

Suitable extinguishing media
Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

5.2 Special hazards arising from the substance or mixture
Carbon oxides, Nitrogen oxides (NOx)

5.3 Advice for firefighters
Wear self-contained breathing apparatus for firefighting if necessary.

5.4 Further information
No data available

6. ACCIDENTAL RELEASE MEASURES

6.1 Personal precautions, protective equipment and emergency procedures
Wear respiratory protection. Avoid dust formation. Avoid breathing vapours, mist or gas. Ensure adequate ventilation. Evacuate personnel to safe areas. Avoid breathing dust.
For personal protection see section 8.

6.2 Environmental precautions
Prevent further leakage or spillage if safe to do so. Do not let product enter drains.

6.3 Methods and materials for containment and cleaning up
Pick up and arrange disposal without creating dust. Sweep up and shovel. Keep in suitable, closed containers for disposal.

6.4 Reference to other sections
For disposal see section 13.

7. HANDLING AND STORAGE

7.1 Precautions for safe handling
Avoid contact with skin and eyes. Avoid formation of dust and aerosols.
Provide appropriate exhaust ventilation at places where dust is formed.
For precautions see section 2.2.

7.2 Conditions for safe storage, including any incompatibilities
Keep container tightly closed in a dry and well-ventilated place.

Recommended storage temperature 2 - 8 °C

Keep in a dry place.

7.3 Specific end use(s)
Apart from the uses mentioned in section 1.2 no other specific uses are stipulated

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

8.1 Control parameters

Components with workplace control parameters
Contains no substances with occupational exposure limit values.

8.2 Exposure controls

Appropriate engineering controls
Avoid contact with skin, eyes and clothing. Wash hands before breaks and immediately after handling the product.

Personal protective equipment

Eye/face protection
Face shield and safety glasses Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).

Skin protection
Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.

Body Protection
Complete suit protecting against chemicals, The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

Respiratory protection
Where risk assessment shows air-purifying respirators are appropriate use a full-face particle respirator type N99 (US) or type P2 (EN 143) respirator cartridges as a backup to engineering controls. If the respirator is the sole means of protection, use a full-face supplied air respirator. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

Control of environmental exposure
Prevent further leakage or spillage if safe to do so. Do not let product enter drains.

9. PHYSICAL AND CHEMICAL PROPERTIES

9.1 Information on basic physical and chemical properties

a)	Appearance	Form: powder Colour: off-white
b)	Odour	No data available
c)	Odour Threshold	No data available
d)	pH	No data available
e)	Melting point/freezing point	No data available
f)	Initial boiling point and boiling range	No data available
g)	Flash point	No data available
h)	Evaporation rate	No data available
i)	Flammability (solid, gas)	No data available
j)	Upper/lower flammability or explosive limits	No data available
k)	Vapour pressure	No data available
l)	Vapour density	No data available
m)	Relative density	No data available
n)	Water solubility	No data available
o)	Partition coefficient: n-octanol/water	No data available
p)	Auto-ignition temperature	No data available
q)	Decomposition temperature	No data available
r)	Viscosity	No data available
s)	Explosive properties	No data available
t)	Oxidizing properties	No data available

9.2 Other safety information
No data available

10. STABILITY AND REACTIVITY

10.1 Reactivity
No data available

10.2 Chemical stability
Stable under recommended storage conditions.

10.3 Possibility of hazardous reactions
No data available

10.4 Conditions to avoid
No data available

10.5 Incompatible materials
Strong oxidizing agents, Strong bases

10.6 Hazardous decomposition products
Other decomposition products - No data available
In the event of fire: see section 5

11. TOXICOLOGICAL INFORMATION

11.1 Information on toxicological effects

Acute toxicity
No data available

Dermal: No data available

No data available

Skin corrosion/irritation
No data available

Serious eye damage/eye irritation
No data available

Respiratory or skin sensitisation
No data available

Germ cell mutagenicity
Hamster
Lungs
Mutation in microorganisms

Carcinogenicity

IARC:	No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.
ACGIH:	No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by ACGIH.
NTP:	No component of this product present at levels greater than or equal to 0.1% is identified as a known or anticipated carcinogen by NTP.
OSHA:	No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by OSHA.

Reproductive toxicity
No data available

No data available

Specific target organ toxicity - single exposure
Inhalation - May cause respiratory irritation.

Specific target organ toxicity - repeated exposure
No data available

Aspiration hazard
No data available

Additional Information
RTECS: RA5998000

prolonged or repeated exposure can cause:, Liver injury may occur., Diarrhoea

Stomach - Irregularities - Based on Human Evidence
Stomach - Irregularities - Based on Human Evidence

12. ECOLOGICAL INFORMATION

12.1 Toxicity
No data available

12.2 Persistence and degradability
No data available

12.3 Bioaccumulative potential
No data available

12.4 Mobility in soil
No data available

12.5 Results of PBT and vPvB assessment
PBT/vPvB assessment not available as chemical safety assessment not required/not conducted

12.6 Other adverse effects

No data available

13. DISPOSAL CONSIDERATIONS

13.1 Waste treatment methods

Product
Offer surplus and non-recyclable solutions to a licensed disposal company. Contact a licensed professional waste disposal service to dispose of this material. Dissolve or mix the material with a combustible solvent and burn in a chemical incinerator equipped with an afterburner and scrubber.

Contaminated packaging
Dispose of as unused product.

14. TRANSPORT INFORMATION

DOT (US)
UN number: 3462 Class: 6.1 Packing group: II
Proper shipping name: Toxins, extracted from living sources, solid, n.o.s. (8-Methyl-N-vanillylnonanamide)
Reportable Quantity (RQ):
Marine pollutant: No
Poison Inhalation Hazard: No

IMDG
UN number: 3462 Class: 6.1 Packing group: II EMS-No: F-A, S-A
Proper shipping name: TOXINS, EXTRACTED FROM LIVING SOURCES, SOLID, N.O.S. (8-Methyl-N-vanillylnonanamide)
Marine pollutant: No

IATA
UN number: 3462 Class: 6.1 Packing group: II
Proper shipping name: Toxins, extracted from living sources, solid, n.o.s. (8-Methyl-N-vanillylnonanamide)

15. REGULATORY INFORMATION

SARA 302 Components
No chemicals in this material are subject to the reporting requirements of SARA Title III, Section 302.

SARA 313 Components
This material does not contain any chemical components with known CAS numbers that exceed the threshold (De Minimis) reporting levels established by SARA Title III, Section 313.

SARA 311/312 Hazards
Acute Health Hazard, Chronic Health Hazard

Massachusetts Right To Know Components
No components are subject to the Massachusetts Right to Know Act.

Pennsylvania Right To Know Components

	CAS-No.	Revision Date
8-Methyl-N-vanillylnonanamide	19408-84-5	

New Jersey Right To Know Components

	CAS-No.	Revision Date
8-Methyl-N-vanillylnonanamide	19408-84-5	

California Prop. 65 Components

This product does not contain any chemicals known to State of California to cause cancer, birth defects, or any other reproductive harm.

16. OTHER INFORMATION

Full text of H-Statements referred to under sections 2 and 3.

Acute Tox.	Acute toxicity
Eye Irrit.	Eye irritation
H301	Toxic if swallowed.
H315	Causes skin irritation.
H319	Causes serious eye irritation.
H335	May cause respiratory irritation.
Skin Irrit.	Skin irritation

HMIS Rating

Health hazard:	2
Chronic Health Hazard:	*
Flammability:	0
Physical Hazard	0

NFPA Rating

Health hazard:	2
Fire Hazard:	0
Reactivity Hazard:	0

Further information

Copyright 2014 Sigma-Aldrich Co. LLC. License granted to make unlimited paper copies for internal use only.
The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Corporation and its Affiliates shall not be held liable for any damage resulting from handling or from contact with the above product. See www.sigma-aldrich.com and/or the reverse side of invoice or packing slip for additional terms and conditions of sale.

Preparation Information

Sigma-Aldrich Corporation
Product Safety – Americas Region
1-800-521-8956

Version: 5.3 Revision Date: 08/27/2014 Print Date: 03/30/2015

SAFETY DATA SHEET

Version 4.5
Revision Date 07/01/2014
Print Date 03/30/2015

1. PRODUCT AND COMPANY IDENTIFICATION

1.1 Product identifiers

Product name : Coumarin

Product Number : C4261
Brand : Sigma

CAS-No. : 91-64-5

1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses : Laboratory chemicals, Manufacture of substances

1.3 Details of the supplier of the safety data sheet

Company : Sigma-Aldrich
3050 Spruce Street
SAINT LOUIS MO 63103
USA

Telephone : +1 800-325-5832
Fax : +1 800-325-5052

1.4 Emergency telephone number

Emergency Phone # : (314) 776-6555

2. HAZARDS IDENTIFICATION

2.1 Classification of the substance or mixture

GHS Classification in accordance with 29 CFR 1910 (OSHA HCS)
Acute toxicity, Oral (Category 3), H301
Acute aquatic toxicity (Category 3), H402
Chronic aquatic toxicity (Category 3), H412

For the full text of the H-Statements mentioned in this Section, see Section 16.

2.2 GHS Label elements, including precautionary statements

Pictogram

Signal word Danger

Hazard statement(s)
H301 Toxic if swallowed.
H412 Harmful to aquatic life with long lasting effects.

Precautionary statement(s)
P264 Wash skin thoroughly after handling.
P270 Do not eat, drink or smoke when using this product.
P273 Avoid release to the environment.
P301 + P310 IF SWALLOWED: Immediately call a POISON CENTER or doctor/ physician.
P321 Specific treatment (see supplemental first aid instructions on this label).
P330 Rinse mouth.
P405 Store locked up.
P501 Dispose of contents/ container to an approved waste disposal plant.

2.3 Hazards not otherwise classified (HNOC) or not covered by GHS - none

3. COMPOSITION/INFORMATION ON INGREDIENTS

3.1 Substances

Synonyms : 1,2-Benzopyrone
1-Benzopyran-2-one
2H-Chromen-2-one

Formula : $C_9H_6O_2$
Molecular Weight : 146.14 g/mol
CAS-No. : 91-64-5
EC-No. : 202-086-7

Hazardous components

Component	Classification	Concentration
Coumarin		
	Acute Tox. 3; Aquatic Acute 3; Aquatic Chronic 3; H301, H412	-

For the full text of the H-Statements mentioned in this Section, see Section 16.

4. FIRST AID MEASURES

4.1 Description of first aid measures

General advice
Consult a physician. Show this safety data sheet to the doctor in attendance.Move out of dangerous area.

If inhaled
If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.

In case of skin contact
Wash off with soap and plenty of water. Take victim immediately to hospital. Consult a physician.

In case of eye contact
Flush eyes with water as a precaution.

If swallowed
Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

4.2 Most important symptoms and effects, both acute and delayed
The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11

4.3 Indication of any immediate medical attention and special treatment needed
no data available

5. FIREFIGHTING MEASURES

5.1 Extinguishing media

Suitable extinguishing media
Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

5.2 Special hazards arising from the substance or mixture
Carbon oxides

5.3 Advice for firefighters
Wear self contained breathing apparatus for fire fighting if necessary.

5.4 Further information
no data available

6. ACCIDENTAL RELEASE MEASURES

6.1 Personal precautions, protective equipment and emergency procedures
Wear respiratory protection. Avoid dust formation. Avoid breathing vapours, mist or gas. Ensure adequate ventilation. Evacuate personnel to safe areas. Avoid breathing dust.
For personal protection see section 8.

6.2 Environmental precautions
Prevent further leakage or spillage if safe to do so. Do not let product enter drains. Discharge into the environment must be avoided.

6.3 Methods and materials for containment and cleaning up
Pick up and arrange disposal without creating dust. Sweep up and shovel. Keep in suitable, closed containers for disposal.

6.4 Reference to other sections
For disposal see section 13.

7. HANDLING AND STORAGE

7.1 Precautions for safe handling
Avoid contact with skin and eyes. Avoid formation of dust and aerosols.
Provide appropriate exhaust ventilation at places where dust is formed.Normal measures for preventive fire protection.
For precautions see section 2.2.

7.2 Conditions for safe storage, including any incompatibilities
Keep container tightly closed in a dry and well-ventilated place.

7.3 Specific end use(s)
Apart from the uses mentioned in section 1.2 no other specific uses are stipulated

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

8.1 Control parameters

Components with workplace control parameters
Contains no substances with occupational exposure limit values.

8.2 Exposure controls

Appropriate engineering controls
Avoid contact with skin, eyes and clothing. Wash hands before breaks and immediately after handling the product.

Personal protective equipment

Eye/face protection
Face shield and safety glasses Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).

Skin protection
Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.

Full contact
Material: Nitrile rubber
Minimum layer thickness: 0.11 mm
Break through time: 480 min
Material tested:Dermatril® (KCL 740 / Aldrich Z677272, Size M)

Splash contact
Material: Nitrile rubber
Minimum layer thickness: 0.11 mm
Break through time: 480 min
Material tested:Dermatril® (KCL 740 / Aldrich Z677272, Size M)

data source: KCL GmbH, D-36124 Eichenzell, phone +49 (0)6659 87300, e-mail sales@kcl.de, test method: EN374

If used in solution, or mixed with other substances, and under conditions which differ from EN 374, contact the supplier of the CE approved gloves. This recommendation is advisory only and must be evaluated by an industrial hygienist and safety officer familiar with the specific situation of anticipated use by our customers. It should not be construed as offering an approval for any specific use scenario.

Body Protection
Complete suit protecting against chemicals, The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

Respiratory protection
Where risk assessment shows air-purifying respirators are appropriate use a full-face particle respirator type N99 (US) or type P2 (EN 143) respirator cartridges as a backup to engineering controls. If the respirator is the sole means of protection, use a full-face supplied air respirator. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

Control of environmental exposure
Prevent further leakage or spillage if safe to do so. Do not let product enter drains. Discharge into the environment must be avoided.

9. PHYSICAL AND CHEMICAL PROPERTIES

9.1 Information on basic physical and chemical properties

a)	Appearance	Form: flakes Colour: colourless
b)	Odour	no data available
c)	Odour Threshold	no data available
d)	pH	no data available
e)	Melting point/freezing point	Melting point/range: 68 - 73 °C (154 - 163 °F) - lit.
f)	Initial boiling point and boiling range	298 °C (568 °F) - lit.
g)	Flash point	no data available
h)	Evapouration rate	no data available
i)	Flammability (solid, gas)	no data available
j)	Upper/lower flammability or explosive limits	no data available
k)	Vapour pressure	no data available
l)	Vapour density	no data available
m)	Relative density	no data available
n)	Water solubility	no data available
o)	Partition coefficient: n-octanol/water	no data available
p)	Auto-ignition temperature	no data available
q)	Decomposition temperature	no data available
r)	Viscosity	no data available
s)	Explosive properties	no data available
t)	Oxidizing properties	no data available

9.2 Other safety information
no data available

10. STABILITY AND REACTIVITY

10.1 Reactivity
no data available

10.2 Chemical stability
Stable under recommended storage conditions.

10.3 Possibility of hazardous reactions
no data available

10.4 Conditions to avoid
no data available

10.5 Incompatible materials
Strong oxidizing agents, Strong acids, Strong bases

10.6 Hazardous decomposition products
Other decomposition products - no data available
In the event of fire: see section 5

11. TOXICOLOGICAL INFORMATION

11.1 Information on toxicological effects

Acute toxicity
LD50 Oral - rat - 293 mg/kg

Inhalation: no data available

Dermal: no data available

no data available

Skin corrosion/irritation
no data available

Serious eye damage/eye irritation
no data available

Respiratory or skin sensitisation
no data available

Germ cell mutagenicity
no data available

Carcinogenicity

This product is or contains a component that is not classifiable as to its carcinogenicity based on its IARC, ACGIH, NTP, or EPA classification.

IARC:	3 - Group 3: Not classifiable as to its carcinogenicity to humans (Coumarin)
ACGIH:	No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by ACGIH.
NTP:	No component of this product present at levels greater than or equal to 0.1% is identified as a known or anticipated carcinogen by NTP.
OSHA:	No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by OSHA.

Reproductive toxicity
no data available

no data available

Specific target organ toxicity - single exposure
no data available

Specific target organ toxicity - repeated exposure
no data available

Aspiration hazard
no data available

Additional Information
RTECS: GN4200000

To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

Stomach - Irregularities - Based on Human Evidence
Stomach - Irregularities - Based on Human Evidence

12. ECOLOGICAL INFORMATION

12.1 Toxicity

Toxicity to fish	LC50 - Poecilia reticulata (guppy) - 56 mg/l - 96 h
Toxicity to daphnia and other aquatic invertebrates	LC50 - Daphnia magna (Water flea) - 13.5 mg/l - 48 h

12.2 Persistence and degradability

12.3 Bioaccumulative potential

Bioaccumulation	Leuciscus idus melanotus - 3 d - 46 µg/l
	Bioconcentration factor (BCF): < 10

12.4 Mobility in soil
no data available

12.5 Results of PBT and vPvB assessment
PBT/vPvB assessment not available as chemical safety assessment not required/not conducted

12.6 Other adverse effects
An environmental hazard cannot be excluded in the event of unprofessional handling or disposal.
Harmful to aquatic life.

no data available

13. DISPOSAL CONSIDERATIONS

13.1 Waste treatment methods

Product
Offer surplus and non-recyclable solutions to a licensed disposal company. Contact a licensed professional waste disposal service to dispose of this material. Dissolve or mix the material with a combustible solvent and burn in a chemical incinerator equipped with an afterburner and scrubber.

Contaminated packaging
Dispose of as unused product.

14. TRANSPORT INFORMATION

DOT (US)
UN number: 2811 Class: 6.1 Packing group: III
Proper shipping name: Toxic solids, organic, n.o.s. (Coumarin)
Marine pollutant: No
Poison Inhalation Hazard: No

IMDG
UN number: 2811 Class: 6.1 Packing group: III EMS-No: F-A, S-A
Proper shipping name: TOXIC SOLID, ORGANIC, N.O.S. (Coumarin)
Marine pollutant: No

IATA
UN number: 2811 Class: 6.1 Packing group: III
Proper shipping name: Toxic solid, organic, n.o.s. (Coumarin)

15. REGULATORY INFORMATION

SARA 302 Components
SARA 302: No chemicals in this material are subject to the reporting requirements of SARA Title III, Section 302.

SARA 313 Components
SARA 313: This material does not contain any chemical components with known CAS numbers that exceed the threshold (De Minimis) reporting levels established by SARA Title III, Section 313.

SARA 311/312 Hazards
Acute Health Hazard, Chronic Health Hazard

Massachusetts Right To Know Components
No components are subject to the Massachusetts Right to Know Act.

Pennsylvania Right To Know Components

	CAS-No.	Revision Date
Coumarin	91-64-5	

New Jersey Right To Know Components

	CAS-No.	Revision Date
Coumarin	91-64-5	

California Prop. 65 Components
This product does not contain any chemicals known to State of California to cause cancer, birth defects, or any other reproductive harm.

16. OTHER INFORMATION

Full text of H-Statements referred to under sections 2 and 3.

Acute Tox.	Acute toxicity
Aquatic Acute	Acute aquatic toxicity
Aquatic Chronic	Chronic aquatic toxicity
H301	Toxic if swallowed.
H402	Harmful to aquatic life.

HMIS Rating

Health hazard:	2
Chronic Health Hazard:	*
Flammability:	0
Physical Hazard	0

NFPA Rating

Health hazard:	2
Fire Hazard:	0
Reactivity Hazard:	0

Further information
Copyright 2014 Sigma-Aldrich Co. LLC. License granted to make unlimited paper copies for internal use only.
The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Corporation and its Affiliates shall not be held liable for any damage resulting from handling

or from contact with the above product. See www.sigma-aldrich.com and/or the reverse side of invoice or packing slip for additional terms and conditions of sale.

Preparation Information
Sigma-Aldrich Corporation
Product Safety – Americas Region
1-800-521-8956

Version: 4.5 Revision Date: 07/01/2014 Print Date: 03/30/2015

SAFETY DATA SHEET

Version 4.7
Revision Date 02/28/2015
Print Date 03/30/2015

1. PRODUCT AND COMPANY IDENTIFICATION

1.1 Product identifiers

Product name : Lead Standard for AAS

Product Number : 16595
Brand : Fluka

1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses : Laboratory chemicals, Manufacture of substances

1.3 Details of the supplier of the safety data sheet

Company : Sigma-Aldrich
3050 Spruce Street
SAINT LOUIS MO 63103
USA

Telephone : +1 800-325-5832
Fax : +1 800-325-5052

1.4 Emergency telephone number

Emergency Phone # : (314) 776-6555

2. HAZARDS IDENTIFICATION

2.1 Classification of the substance or mixture

GHS Classification in accordance with 29 CFR 1910 (OSHA HCS)
Skin irritation (Category 2), H315
Serious eye damage (Category 1), H318
Carcinogenicity (Category 1B), H350
Reproductive toxicity (Category 1A), H360
Acute aquatic toxicity (Category 3), H402
Chronic aquatic toxicity (Category 3), H412

For the full text of the H-Statements mentioned in this Section, see Section 16.

2.2 GHS Label elements, including precautionary statements

Pictogram

Signal word Danger

Hazard statement(s)
H315 Causes skin irritation.
H318 Causes serious eye damage.
H350 May cause cancer.
H360 May damage fertility or the unborn child.
H412 Harmful to aquatic life with long lasting effects.

Precautionary statement(s)
P201 Obtain special instructions before use.
P202 Do not handle until all safety precautions have been read and understood.
P264 Wash skin thoroughly after handling.

P273	Avoid release to the environment.
P280	Wear protective gloves/ protective clothing/ eye protection/ face protection.
P302 + P352	IF ON SKIN: Wash with plenty of soap and water.
P305 + P351 + P338 + P310	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER or doctor/ physician.
P308 + P313	IF exposed or concerned: Get medical advice/ attention.
P332 + P313	If skin irritation occurs: Get medical advice/ attention.
P362	Take off contaminated clothing and wash before reuse.
P405	Store locked up.
P501	Dispose of contents/ container to an approved waste disposal plant.

2.3 Hazards not otherwise classified (HNOC) or not covered by GHS - none

3. COMPOSITION/INFORMATION ON INGREDIENTS

3.2 Mixtures

Hazardous components

Component		Classification	Concentration
Nitric acid			
CAS-No. EC-No. Index-No.	7697-37-2 231-714-2 007-004-00-1	Ox. Liq. 3; Skin Corr. 1A; Eye Dam. 1; H272, H314	>= 1 - < 5 %
Lead nitrate Included in the Candidate List of Substances of Very High Concern (SVHC) according to Regulation (EC) No. 1907/2006 (REACH)			
CAS-No. EC-No. Index-No.	10099-74-8 233-245-9 082-001-00-6	Ox. Sol. 2; Acute Tox. 4; Eye Dam. 1; Carc. 1B; Repr. 1A; STOT RE 2; Aquatic Acute 1; Aquatic Chronic 1; H272, H302 + H332, H318, H350, H360, H373, H410	>= 0.1 - < 1 %

For the full text of the H-Statements mentioned in this Section, see Section 16.

4. FIRST AID MEASURES

4.1 Description of first aid measures

General advice
Consult a physician. Show this safety data sheet to the doctor in attendance.Move out of dangerous area.

If inhaled
If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.

In case of skin contact
Wash off with soap and plenty of water. Consult a physician.

In case of eye contact
Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician.Continue rinsing eyes during transport to hospital.

If swallowed
Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

4.2 Most important symptoms and effects, both acute and delayed
The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11

4.3 Indication of any immediate medical attention and special treatment needed
No data available

5. FIREFIGHTING MEASURES

5.1 Extinguishing media

Suitable extinguishing media
Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

5.2 Special hazards arising from the substance or mixture
Nitrogen oxides (NOx)

5.3 Advice for firefighters
Wear self-contained breathing apparatus for firefighting if necessary.

5.4 Further information
No data available

6. ACCIDENTAL RELEASE MEASURES

6.1 Personal precautions, protective equipment and emergency procedures
Use personal protective equipment. Avoid breathing vapours, mist or gas. Ensure adequate ventilation. Evacuate personnel to safe areas.
For personal protection see section 8.

6.2 Environmental precautions
Prevent further leakage or spillage if safe to do so. Do not let product enter drains. Discharge into the environment must be avoided.

6.3 Methods and materials for containment and cleaning up
Soak up with inert absorbent material and dispose of as hazardous waste. Keep in suitable, closed containers for disposal.

6.4 Reference to other sections
For disposal see section 13.

7. HANDLING AND STORAGE

7.1 Precautions for safe handling
Avoid contact with skin and eyes. Avoid inhalation of vapour or mist.
For precautions see section 2.2.

7.2 Conditions for safe storage, including any incompatibilities
Keep container tightly closed in a dry and well-ventilated place. Containers which are opened must be carefully resealed and kept upright to prevent leakage.
Storage class (TRGS 510): Non Combustible Liquids

7.3 Specific end use(s)
Apart from the uses mentioned in section 1.2 no other specific uses are stipulated

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

8.1 Control parameters

Components with workplace control parameters

Component	CAS-No.	Value	Control parameters	Basis
Nitric acid	7697-37-2	TWA	2.000000 ppm	USA. ACGIH Threshold Limit Values (TLV)
	Remarks	Upper Respiratory Tract irritation Eye irritation Dental erosion		
		STEL	4.000000 ppm	USA. ACGIH Threshold Limit Values (TLV)
		Upper Respiratory Tract irritation Eye irritation Dental erosion		

		ST	4.000000 ppm 10.000000 mg/m3	USA. NIOSH Recommended Exposure Limits
		TWA	2.000000 ppm 5.000000 mg/m3	USA. NIOSH Recommended Exposure Limits
		TWA	2.000000 ppm 5.000000 mg/m3	USA. Occupational Exposure Limits (OSHA) - Table Z-1 Limits for Air Contaminants
		The value in mg/m3 is approximate.		
Lead nitrate	10099-74-8	TWA	0.050000 mg/m3	USA. ACGIH Threshold Limit Values (TLV)
		Central Nervous System impairment Hematologic effects Peripheral Nervous System impairment Substances for which there is a Biological Exposure Index or Indices (see BEI® section) Confirmed animal carcinogen with unknown relevance to humans varies		
		PEL	0.050000 mg/m3	OSHA Specifically Regulated Chemicals/Carcinogens
		1910.1025 If an employee is exposed to lead for more than 8 hours in any work day, the permissible exposure limit, as a time weighted average (TWA) for that day, shall be reduced according to the following formula: Maximum permissible limit (in µg/m3)=400÷hours worked in the day This section applies to all occupational exposure to lead, except as provided in paragraph (a)(2). It does not apply to the construction industry or to agricultural operations covered by 29 CFR part 1928. OSHA specifically regulated carcinogen		
		TWA	0.050000 mg/m3	USA. NIOSH Recommended Exposure Limits
		See Appendix C		
		PEL	0.050000 mg/m3	OSHA Specifically Regulated Chemicals/Carcinogens
		1910.1025 If an employee is exposed to lead for more than 8 hours in any work day, the permissible exposure limit, as a time weighted average (TWA) for that day, shall be reduced according to the following formula: Maximum permissible limit (in µg/m3)=400÷hours worked in the day This section applies to all occupational exposure to lead, except as provided in paragraph (a)(2). It does not apply to the construction industry or to agricultural operations covered by 29 CFR part 1928. OSHA specifically regulated carcinogen		
		TWA	0.05 mg/m3	USA. ACGIH Threshold Limit Values (TLV)
		Central Nervous System impairment Hematologic effects Peripheral Nervous System impairment Substances for which there is a Biological Exposure Index or Indices (see BEI® section) Confirmed animal carcinogen with unknown relevance to humans varies		
		PEL	0.05 mg/m3	OSHA Specifically Regulated Chemicals/Carcinogens
		1910.1025 If an employee is exposed to lead for more than 8 hours in any work day, the permissible exposure limit, as a time weighted average		

		(TWA) for that day, shall be reduced according to the following formula: Maximum permissible limit (in µg/m3)=400÷hours worked in the day This section applies to all occupational exposure to lead, except as provided in paragraph (a)(2). It does not apply to the construction industry or to agricultural operations covered by 29 CFR part 1928. OSHA specifically regulated carcinogen		
		TWA	0.05 mg/m3	USA. NIOSH Recommended Exposure Limits
		See Appendix C		

8.2 Exposure controls

Appropriate engineering controls
Handle in accordance with good industrial hygiene and safety practice. Wash hands before breaks and at the end of workday.

Personal protective equipment

Eye/face protection
Tightly fitting safety goggles. Faceshield (8-inch minimum). Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).

Skin protection
Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.

Body Protection
Complete suit protecting against chemicals, The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

Respiratory protection
Where risk assessment shows air-purifying respirators are appropriate use a full-face respirator with multi-purpose combination (US) or type ABEK (EN 14387) respirator cartridges as a backup to engineering controls. If the respirator is the sole means of protection, use a full-face supplied air respirator. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

Control of environmental exposure
Prevent further leakage or spillage if safe to do so. Do not let product enter drains. Discharge into the environment must be avoided.

9. PHYSICAL AND CHEMICAL PROPERTIES

9.1 Information on basic physical and chemical properties

a)	Appearance	Form: clear, liquid Colour: colourless
b)	Odour	No data available
c)	Odour Threshold	No data available
d)	pH	< 1.0
e)	Melting point/freezing point	No data available
f)	Initial boiling point and boiling range	No data available
g)	Flash point	No data available
h)	Evaporation rate	No data available
i)	Flammability (solid, gas)	No data available
j)	Upper/lower flammability or explosive limits	No data available

k)	Vapour pressure	No data available
l)	Vapour density	No data available
m)	Relative density	1.020 g/cm3
n)	Water solubility	No data available
o)	Partition coefficient: n-octanol/water	No data available
p)	Auto-ignition temperature	No data available
q)	Decomposition temperature	No data available
r)	Viscosity	No data available
s)	Explosive properties	No data available
t)	Oxidizing properties	No data available

9.2 Other safety information
No data available

10. STABILITY AND REACTIVITY

10.1 Reactivity
No data available

10.2 Chemical stability
Stable under recommended storage conditions.

10.3 Possibility of hazardous reactions
No data available

10.4 Conditions to avoid
Light.

10.5 Incompatible materials
Alkali metals, Aluminum, Amines, Bases, Copper

10.6 Hazardous decomposition products
Other decomposition products - No data available
In the event of fire: see section 5

11. TOXICOLOGICAL INFORMATION

11.1 Information on toxicological effects

Acute toxicity
No data available

Inhalation: No data available

Dermal: No data available

No data available

Skin corrosion/irritation
No data available

Serious eye damage/eye irritation
No data available

Respiratory or skin sensitisation
No data available

Germ cell mutagenicity
No data available

Carcinogenicity

IARC: 2A - Group 2A: Probably carcinogenic to humans (Lead nitrate)

2A - Group 2A: Probably carcinogenic to humans (Lead nitrate)

NTP: Reasonably anticipated to be a human carcinogenThe reference note has been added by TD based on the background information of the NTP. (Lead nitrate)

OSHA: OSHA specifically regulated carcinogen (Lead nitrate)

Reproductive toxicity
No data available
No data available

Specific target organ toxicity - single exposure
No data available

Specific target organ toxicity - repeated exposure
No data available

Aspiration hazard
No data available

Additional Information
RTECS: Not available

To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

Liver - Irregularities - Based on Human Evidence
Stomach - Irregularities - Based on Human Evidence
Liver - Irregularities - Based on Human Evidence (Nitric acid)
Stomach - Irregularities - Based on Human Evidence (Lead nitrate)

12. ECOLOGICAL INFORMATION

12.1 Toxicity
No data available

12.2 Persistence and degradability
No data available

12.3 Bioaccumulative potential
No data available

12.4 Mobility in soil
No data available

12.5 Results of PBT and vPvB assessment
PBT/vPvB assessment not available as chemical safety assessment not required/not conducted

12.6 Other adverse effects
An environmental hazard cannot be excluded in the event of unprofessional handling or disposal.
Harmful to aquatic life with long lasting effects.

13. DISPOSAL CONSIDERATIONS

13.1 Waste treatment methods

Product
Offer surplus and non-recyclable solutions to a licensed disposal company. Contact a licensed professional waste disposal service to dispose of this material. Dissolve or mix the material with a combustible solvent and burn in a chemical incinerator equipped with an afterburner and scrubber.

Contaminated packaging
Dispose of as unused product.

14. TRANSPORT INFORMATION

DOT (US)

UN number: 3264 Class: 8 Packing group: III
Proper shipping name: Corrosive liquid, acidic, inorganic, n.o.s. (Nitric acid)
Reportable Quantity (RQ): 5000 lbs

Poison Inhalation Hazard: No

IMDG
UN number: 3264 Class: 8 Packing group: III EMS-No: F-A, S-B
Proper shipping name: CORROSIVE LIQUID, ACIDIC, INORGANIC, N.O.S. (Nitric acid)

IATA
UN number: 3264 Class: 8 Packing group: III
Proper shipping name: Corrosive liquid, acidic, inorganic, n.o.s. (Nitric acid)

15. REGULATORY INFORMATION

SARA 302 Components
The following components are subject to reporting levels established by SARA Title III, Section 302:

	CAS-No.	Revision Date
Nitric acid	7697-37-2	2007-07-01

SARA 313 Components
The following components are subject to reporting levels established by SARA Title III, Section 313:

	CAS-No.	Revision Date
Nitric acid	7697-37-2	2007-07-01

Massachusetts Right To Know Components

	CAS-No.	Revision Date
Nitric acid	7697-37-2	2007-07-01

Pennsylvania Right To Know Components

	CAS-No.	Revision Date
Water	7732-18-5	
Nitric acid	7697-37-2	2007-07-01
Lead nitrate	10099-74-8	1993-04-24

New Jersey Right To Know Components

	CAS-No.	Revision Date
Water	7732-18-5	
Nitric acid	7697-37-2	2007-07-01
Lead nitrate	10099-74-8	1993-04-24

California Prop. 65 Components

WARNING! This product contains a chemical known to the State of California to cause cancer.	CAS-No.	Revision Date
Lead nitrate	10099-74-8	2007-09-28

16. OTHER INFORMATION

Full text of H-Statements referred to under sections 2 and 3.

Acute Tox.	Acute toxicity
Aquatic Acute	Acute aquatic toxicity
Aquatic Chronic	Chronic aquatic toxicity
Carc.	Carcinogenicity
Eye Dam.	Serious eye damage
H272	May intensify fire; oxidiser.
H302 + H332	Harmful if swallowed or if inhaled
H314	Causes severe skin burns and eye damage.
H315	Causes skin irritation.
H318	Causes serious eye damage.

H350	May cause cancer.
H360	May damage fertility or the unborn child.
H373	May cause damage to organs through prolonged or repeated exposure.
H402	Harmful to aquatic life.
H410	Very toxic to aquatic life with long lasting effects.
H412	Harmful to aquatic life with long lasting effects.
Ox. Liq.	Oxidizing liquids
Ox. Sol.	Oxidizing solids
Repr.	Reproductive toxicity
Skin Corr.	Skin corrosion
STOT RE	Specific target organ toxicity - repeated exposure

HMIS Rating

Health hazard:	3
Chronic Health Hazard:	*
Flammability:	0
Physical Hazard	2

NFPA Rating

Health hazard:	3
Fire Hazard:	0
Reactivity Hazard:	0

Further information

Copyright 2015 Sigma-Aldrich Co. LLC. License granted to make unlimited paper copies for internal use only.
The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Corporation and its Affiliates shall not be held liable for any damage resulting from handling or from contact with the above product. See www.sigma-aldrich.com and/or the reverse side of invoice or packing slip for additional terms and conditions of sale.

Preparation Information

Sigma-Aldrich Corporation
Product Safety – Americas Region
1-800-521-8956

Version: 4.7 Revision Date: 02/28/2015 Print Date: 03/30/2015