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The Photodegradation of 2,6-dichloro-4-nitroaniline (DCNA) in Freshwater and Saltwater

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THE PHOTODEGRADATION OF 2,6-DICHLORO-4-NITROANILINE (DCNA) IN FRESHWATER AND SALTWATER

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
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in

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ABSTRACT

      The fungicide 2,6-dichloro-4-nitroaniline (DCNA) is applied to crops grown in areas near both freshwater and saltwater bodies and it can enter the surface waters where it is susceptible to photolysis; limited information is published on the photodegradation of DCNA. It has been shown that the salinity of seawater can influence both the overall rate of degradation of chemicals and impact the distribution and types of photoproducts generated during the photodegradation processes of a pesticide. The photodegradation of DCNA was measured in distilled water, artificial seawater, estuarine water, and phosphate buffer to determine the degree of differences in the degradation rate in various matrices. The brominated analogue 2,6-dibromo-4-nitroaniline (DBNA) was measured identically to determine the impacts of other halogens on the degradation process. Solutions of DCNA and DBNA at a concentration of 1 ppm were prepared and irradiated for 24 hours in an Atlas SUNTEST XXL+ photochamber that mimics the wavelength distribution and intensity of sunlight. Dark controls were run simultaneously. Samples were withdrawn at 0, 2, 4, 6, 12, and 24 hours and analyzed for residual DCNA or DBNA using an Agilent 1260 Infinity High Performance Liquid Chromatograph. The formation of ions such as nitrate, nitrite, bromide, and chloride were measured using a Thermo Dionex ICS-5000+ Ion Chromatograph. The half-life of DCNA in distilled water was calculated to be 7.62 ± 0.094 hours and 7.37 ± 0.279 hours in artificial seawater; statistically there was no significant difference in the degradation rate through the first half-life. Analysis of the quick formation of nitrite and chloride ions, and later formation of nitrate ions, suggests photonucleophilic substitution processes are occurring as the compound is degrading, followed by further degradation of nitrite to nitrate likely also due to photolysis processes. Small aliphatic acids, maleic and fumaric acid, were detected after 12 hours of

irradiation by HPLC indicating degradation of the aromatic ring structure. Differences in formation rate and decline for intermediate photoproducts were observed in seawater and distilled water suggesting salinity affects the rate of formation of this photoproduct.

CHAPTER 1: INTRODUCTION

The fungicide 2,6-dichloro-4-nitroaniline (DCNA) is primarily used to prevent fungal spore germination on various crops, including specialty crops such as stone fruits and celery [1]. It is the active ingredient in the fungicide Botran®, registered and trademarked by Gowan Company, LLC. DCNA is commonly used in areas of coastal California as a means of preventing the growth and spread of fungi such as *Sclerotinia sclerotiorum*, otherwise known as pink rot, which can result in up to a 50% loss in crop yield. DCNA is registered for use on specialty crops like lettuce, celery, and grapes which are grown in coastal areas such as Monterey County, Santa Barbara County, and San Luis Obispo County in California and also in Florida. The location of these crops within coastal areas suggests that DCNA may enter marine and estuarine ecosystems.

DCNA is applied to crops by sprinkler irrigation, areal spray, chemigation, or dip tanks [1]. It has the opportunity to enter surface waters near application sites due the runoff or as drift from the application methods. Because the fungicide can enter surface waters, of both natural freshwater and saltwater bodies, it is likely susceptible to degradation by sunlight.

Previous research has shown that the salinity of the water can affect the degradation rate and half-life of a chemical [2]. The environmental fate of a chemical is highly dependent upon the major degradation processes and the environment in which it is found. Lab studies typically use buffered distilled water for photolysis experiments, which does not take into account the potential affects salinity may have on the photodegradation of a chemical when it enters marine or estuarine waters as opposed to freshwater.

Research on pesticides such as pentachlorophenol (PCP), 3,4-dichloroaniline (DCA), and hydroxychlorothalonil suggest that photonucleophilic substitution can be a major degradation

process in water for chemicals that are susceptible to this process. PCP and DCA were both

analyzed in buffered, distilled water and artificial seawater, (Instant Ocean®), to measure the

difference in degradation rates [2]. In artificial seawater a longer half-life was reported for PCP

than measured in distilled water however a similar half-life was measured for DCA in both

distilled water and artificial seawater. While PCP appeared to be affected by the salinity of the

water, DCA did not leading to the conclusion that the degradation of a compound in freshwater

and seawater is unique to the characteristics of a particular compound.

Limited information on the photodegradation of DCNA is published in literature. The

purpose of this investigation was to determine if there is a difference in photodegradation rate for

DCNA in seawater versus freshwater. Since DCNA is often used in coastal areas, especially

central and southern coastal California and areas in Florida, determining if it degrades differently

in marine ecosystems can be important in assessing if there is a risk to marine organisms. If it

does degrade differently in seawater then in studies typically submitted by pesticide registrants in

support of pesticide registration or reregistration that simulate freshwater ecosystems, the risk of

exposure to marine organisms may be over or underestimated.

CHAPTER 2: LITERATURE REVIEW

2.1.   Salinity and Seawater

The salinity of water plays a key role in the world's aquatic chemistry, especially when one considers that 97% of water on Earth is saline water [3]. Roughly 90% of the ocean has a salinity value ranging from 34.3 ppt to 35.1 ppt; the mean temperature of the world's ocean is $3.25°C$ with a mean salinity of 34.72 ppt. The major ions within surface seawater include, Na^+, K^+, Mg^{++}, Ca^{++}, Cl^-, Sr^{++}, F^-, Br^-, $SO_4^=$, HCO_3^-, and $B(OH)_3$ with trace amounts of the elements cadmium, cobalt, copper, and cesium. Silicon is a minor constituent of seawater; bound to oxygen, it is given the name silica and is an important nutrient and cycle within seawater. Organisms that reside in seawater primarily use calcium carbonate to make shells, but silica is a minor constituent as well.

Oceans are not the only saline waters of the world; estuaries typically have lower salinity values than the oceans due to the mixing of freshwater rivers and the coastal ocean waters. Estuaries are, in part, classified by their salinity gradient; it is dependent upon the amount of freshwater input to the estuary and the tidal and wave action that is mixing freshwater with the input of seawater [4]. Estuaries, deltas, and lagoons are classified by river, wave, and tidal inputs which correlates with the freshwater and seawater gradient or stratification. The salinity gradient of these coastal areas and estuaries can range from 0 ppt due to the input of freshwater to 35 ppt due to the input of ocean water. The salinity within estuaries also varies temporally as a result of tidal action [4].

2.2.   Photonucleophilic Substitution

Salinity has been shown to dramatically impact certain photodegradation processes, especially those involving photonucleophilic substitution. For example, pentachlorophenol

(PCP) showed a difference in half-life in distilled water (with buffer) compared to artificial seawater [2]. PCP in distilled water was reported to have a half-life of 0.9 hours and in artificial seawater a half-life of 2.3 hours. However this trend does not appear to apply uniformly to all chemicals; in the same study Miille and Crosby reported that 3,4-dichloroaniline (DCA) degraded with a half-lives of 17.2 hours and 17.3 hours respectively in distilled water and artificial seawater. This apparent disparity in the effects that seawater has on a chemical's photodegradation process indicates the importance of substituent effects on degradation and also that chemical degradation in response salinity is unique to each chemical.

Pentachlorophenol has been reported to undergo photonucleophilic substitution as a result of the photodegradation process. In this process the chlorine groups on the PCP ring are replaced by hydroxyl groups occurring at the *meta* position to the phenolic moiety on the ring, resulting in various measureable degradation products [2, 5, 6]. DCA also undergoes photonucleophilic substitution; the substitution has been reported to favor the *meta* position, just as PCP, as the first site for the substitution of the chlorine for a hydroxyl group [6]. A photoproduct of the degradation of DCA is 2-chloro-5-aminophenol; supporting that the first substitution of hydroxyl for chlorine occurs at the *meta* position. Photonucleophilic substitution was confirmed when DCA and PCP were irradiated in a solution of 0.6 M Na^{36}Cl. For PCP, 42% of the original compound was radiolabeled with ^{36}Cl; for DCA, no ^{36}Cl was detected [2]. The exchange of chlorine ions appears to be unique to the chlorine-containing compound that is undergoing the degradation reaction within the seawater media

Other chlorine containing compounds that have been reported to undergo photonucleophilic substitution of the ring chlorine for hydroxyl groups include the herbicides 2,4-dichloro-phenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), 3,4-

dichloropropionanilide (propanil), and *p*-chlorophenoxyacetic acid (4-CPA) [7, 8, 9, 10]. Crosby also analyzed the photodegradation of halogenated benzoic acids, both chlorinated and brominated [11]. It was observed that hydroxyl groups, replace both chlorine and bromine on the ring; therefore photonucleophilic substitution can occur in differing halogenated compounds.

The herbicide 2,4-dichlorophenyl-*p*-nitrophenyl ether (nitrofen) contains both chlorine and nitro groups. Photodegradation products of nitrofen reported photonucleophilic substitution of both ring chlorine and nitro groups, which were displaced by hydroxyl groups [12]. Nakagawa and Crosby reported *p*-nitrophenol and hydroquinone as two photoproducts within the degradation pathway of nitrofen, supporting the report of photonucleophilic substitution of the nitro group for hydroxyl.

Hydroxychlorothalonil, a degradation product of the fungicide chlorothalonil, in soil, has been measured in golf course leachate and photodegradation is principally responsible for its dissipation in aquatic systems [13]. Armbrust used distilled water, sterilized pond water, a buffer solution, and salt solutions to determine the degradation products and rates of hydroxychlorothalonil. The compound quickly degraded; ultimately losing all chlorine and cyano groups from the chain structure and forming small products, as shown in Figure 2.1. In the presence of salts, the degradation rate of hydroxychlorothalonil was reported to be slower than without salts, this is similar to the degradation results of pentachlorophenol in seawater [2]. Photonucleophilic substitution appears to be an important degradation process for pentachlorophenol, 3,4-dichloroaniline, and hydroxychlorothalonil [2, 13].
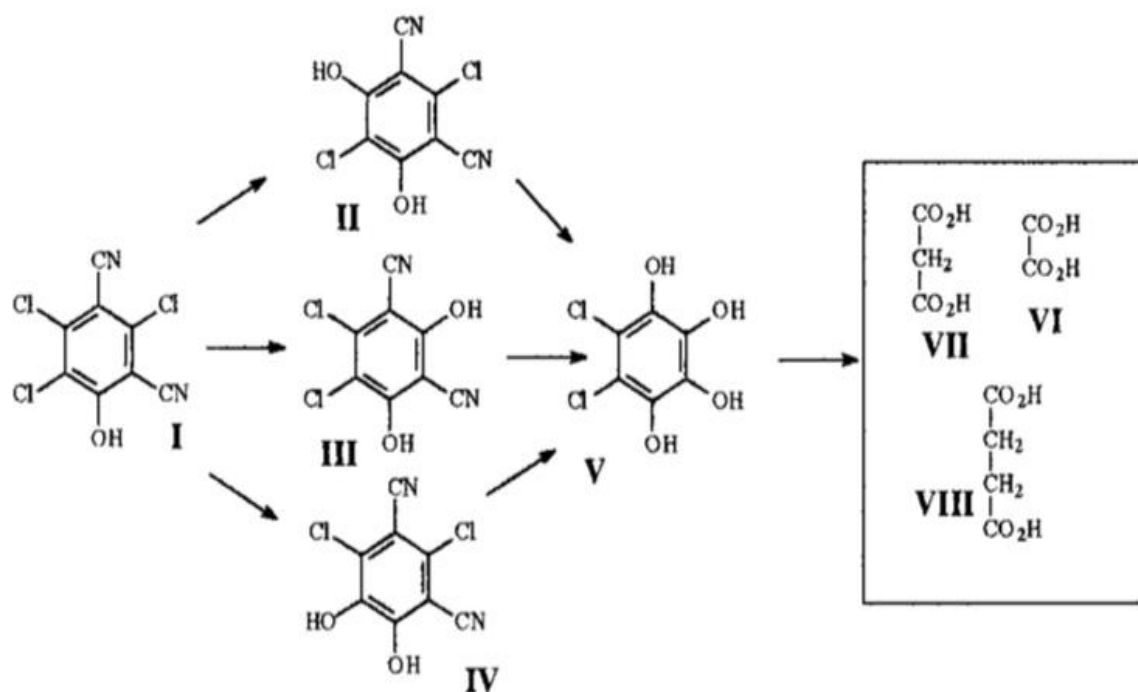
Figure 2.1. The degradation pathway of hydroxychlorothalonil. The degradation pathway includes the aliphatic acids [13].

Previous studies suggest that the degradation of certain pesticides such as hydroxychlorothalonil and pentachlorophenol ultimately result in the formation of small aliphatic acids such as maleic acid, succinic acid, oxalic acid, and fumaric acid [2, 13, 14]. These small acids are commonly formed during the photodegradation of compounds containing nitro or chlorinated ring structures and ultimately result in the degradation of the aromatic ring [13].

2.3.   Nitrite, Nitrate, and Dissolved Nitrogen in Seawater

The formation and degradation of nitrate and nitrite as a result of photolysis is particularly important. Photonucleophilic substitution processes that occur with compounds that have a nitro group on their ring structure commonly result in the nitro group being substituted by a hydroxyl group [15, 16]. This leaves a displaced nitro group which can be measured as nitrite

by ion chromatography [17]. The photolysis of nitrate and nitrite is not limited to distilled water lab conditions or freshwater samples, it is important in seawater as well.

The formation of nitrite from nitrate has been observed in seawater, as well as in laboratory samples in pure water via thermal reactions [18, 19]. In seawater, the regeneration of nitrate and nitrite ions seemingly takes a different route than it takes in pure water and the ions more than likely originate from photosynthetic processes of marine organisms.

Nitrite is an intermediate in the nitrogen cycle, which is particularly important in aquatic systems [18]. Nitrate and nitrite are both subject to photolysis in seawater, which often results in the formation of hydroxyl radicals; the photolysis of nitrite and nitrate is shown in Figure 2.2 [18, 19, 20].

$$NO_2^- + h\nu \rightarrow NO_3^-$$

$$NO_3^- + h\nu \rightarrow NO_2^- + \tfrac{1}{2} O_2$$
$$H_2O + NO_3^- + h\nu \rightarrow NO_2 + \bullet OH + OH^-$$

$$NO_2^- + h\nu \rightarrow *NO_2^-$$
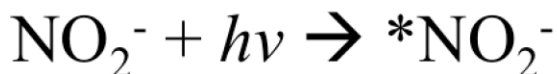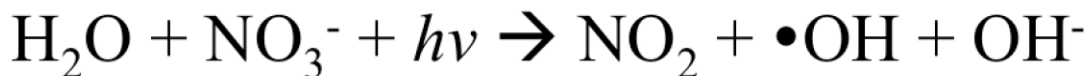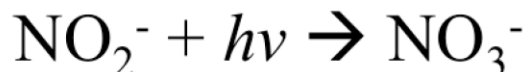$$*NO_2^- + H_2O \rightarrow NO + \bullet OH + OH^-$$

Figure 2.2. Nitrite and nitrate photolysis reactions. The reactions commonly form hydroxyl radicals in water [18, 19].

Hydroxyl radicals are the most reactive of the free radicals produced in natural waters, both freshwater and seawater [21]. The free radicals within the water react with "scavengers," such as bromine ions in seawater or other organic pollutants that also can be found in both freshwater and seawater. Hydroxyl radical concentrations measured in seawater are lower than that measured in freshwater samples due to the reaction of bromine ions and the free radicals in seawater [20].

The production of nitrates and nitrites from fertilizer and pesticide degradation contribute to the nitrogen in these systems; therefore understanding the ways in which nitrate and nitrite act in seawater compared to freshwater is imperatively important. Nitrogen is an important nutrient in both freshwater and seawater. In marine systems, nitrogen is usually the limiting nutrient preventing algal growth [22]. While nitrogen is an important nutrient to living things there has been an increase in the global nitrogen flux particularly due to human causes, which includes the high use of pesticides throughout the world. The reaction between oxygen and nitrogen gas typically results in nitric acid, which dissociates to nitrate in natural waters and makes up the majority of the fixed nitrogen found in seawater [3].

2.4.   Photoproducts

In both the degradation PCP and DCA, a difference in the measured amount of photoproduct in distilled water, Instant Ocean®, and seawater solutions were observed [2]. The major degradation product of PCP is tetrachlorophenol; DCA is 3-chloroaniline. In distilled water, various isomers of tetrachlorophenol were measured to be 0.8% of the photoproduct, while in seawater it was measured to be 6.1%; 3-chlorophenol showed similar results of 2.0% in distilled water and 5.0% in seawater [2]. While it is known that photonucleophilic substitution is responsible for the degradation of these products, the changes in the amount of detectable

photoproduct in different media suggests that salinity can play a role in not just the rate of degradation but also the rate of formation and the amount of photoproduct formed. This aspect becomes highly relevant if different amounts or types of these photoproducts are formed in marine systems that are toxic to marine organisms.

2.5.   Irradiation in Aquatic Systems

Obviously, light of appropriate wavelengths must be present for photodegradation to occur, however light does get attenuated by natural waterbodies depending upon the characteristics of the water. A significant amount of the radiation striking waterbodies of the earth's surface is reflected, however a significant portion penetrates water as well. In the open ocean, 73% of the surface light penetrates the first 1 cm of water. This is reduced to 44.5% at 1 meter and to 22.2% at 10 meters. By 100 m only 0.53% of surface light is available. Near shore, much of the surface light is absorbed within the first few meters of ocean water [23].

Sunlight differentially penetrates water based upon wavelength and the depth of penetration is dependent upon the clarity of the water. Certain wavelengths of light are absorbed before others. Red light is absorbed within the first 5-10 m, orange light is absorbed between 10-15 m, yellow is absorbed between 15-25 m, green is absorbed between 30-50 m, blue is absorbed between 60-100 m, and violet light is absorbed within 10-30 m [24].

Light penetration is unique to the type of water body, streams in the Pacific Northwest do not receive much light due to the dense forests, larger streams of higher stream orders receive more light because there is typically less tree cover, and large rivers, which are typically fairly turbulent, have some surface light penetration but the light does not reach far depths [25].

2.6.   DCNA as a Fungicide

Botran® is registered to Gowan Company, LLC since 1993 [26]. DCNA is the active ingredient in the fungicide Botran 5F®, and is used for controlling *Sclerotinia, Botrytis*, and *Botryospaeria sp.* [27, 28]. Boots Co., Ltd, now Bayer CropScience, first introduced the fungicide before being trademarked and registered with Gowan [26]. Botran® is registered for use in western states such as California, Washington, Idaho, and Oregon; southern states such as Arizona, Texas, Oklahoma, Louisiana, Arkansas, Missouri, Mississippi, Alabama, Tennessee, Kentucky, Georgia, North Carolina, and Florida; and Hawaii. It is currently not pending registration in any other states. Botran® is used on crops such as lettuce, sweet potatoes, onions, garlic, and celery, and it is registered for use on grapes west of the Rocky Mountains.

In 1961, DCNA was registered for use in the United States [1]. A registration standard was completed in 1983 and the Environmental Protection Agency (EPA) issued a Reregistration Eligibility Decision (RED) in 2006. Throughout the United States, over 200,000 pounds ai of DCNA is applied annually. DCNA has a low percent of crop treated for all crops except for celery; 40-60% of celery is treated with DCNA. The maximum application rate for DCNA is 4 lb ai/acre per year for all crops with the exception of potatoes (7.5 lb ai/acre/year) and celery and fennel (5 lb ai/acre/year) [1].

In the United States, celery is grown in California, Texas, Florida, and Michigan. Roughly 75% of the celery grown in the United States is grown in California where it is grown year-round. Over 98% of the celery grown in California is within central and southern coastal regions. Celery is susceptible to pink rot, *Sclerotinia sclerotiorum*, especially in coastal areas and this can cause significant loss of crop yield (5-50%). Crater rot, *Rhizoctonia solani,* also affects celery in coastal California; this fungus is long-lived in soil and can result in 100% loss of

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crop. Botran® is used for the control of pink rot and crater rot in coastal California on celery plants.   Chlorothalonil is the primary fungicide used to control both pink rot and crater rot on celery; DCNA is used in significant quantities, 44,841 lb ai applied to crops in 1997, as a secondary fungicide in controlling the fungi [29].

United States Department of Agriculture (USDA) reports pesticide usage on basis of crop distribution, state, and year through the National Agriculture Statistics Service, however specific amounts of pesticides used are not reported in this system [30]. The State of California has an extensive system for reporting pesticide usage by location and amount. Data obtained from these sites includes an unlisted amount of DCNA applied to tangerines in Florida in 2011. In 1997 in North California 30,800 lbs of DCNA was applied and 10,900 lbs applied in 1999. This number decreased from 1997 to 2005; in 2005, Northern California applied 4,100 lbs of DCNA to grapes and 5,100 lbs of DCNA to grapes throughout the state. DCNA is also applied to head lettuce in California; in 1994 20,400 lbs of DCNA was applied to lettuce as opposed to the 5,200 lbs applied in 2005, and much of the lettuce crops were located within the central and southern coastal areas of California [30].

Figure 2.3 is a map obtained from the California Farm Bureau Federation of various areas in California showing the crops that are grown in different regions. This figure shows the major crops grown in different areas of California. In many of the coastal counties, crops that DCNA is approved for use on are grown; including celery, grapes, and lettuce [31].

California produces...

1 — Timber, Milk, Beef Cattle, Nursery Plants—Lily Bulbs & Woody Ornamentals, Pasture & Rangeland

2 — Timber, Beef Cattle, Alfalfa Hay, Strawberry Plants, Potatoes, Pasture & Rangeland, Wheat

3 — Timber, Beef Cattle, Hay, Strawberry Plants, Pasture & Rangeland, Nursery Plants, Wild Rice

4 — Rice, Almonds, Walnuts, Milk, Dried Plums, Olives, Beef Cattle, Timber

5 — Grapes, Timber, Pears, Beef Cattle, Walnuts, Nursery Plants, Milk

6 — Rice, Almonds, Tomatoes, Walnuts, Beef Cattle, Vegetable Seed, Dried Plums, Peaches

7 — Timber, Rice, Beef Cattle, Grapes, Apples, Pasture & Rangeland, Nursery Plants

8 — Grapes, Tomatoes, Rice, Hay, Nursery Plants, Pears, Almonds, Milk

9 — Grapes, Milk, Beef Cattle, Nursery Plants, Poultry, Pasture & Rangeland, Sheep

10 — Nursery Plants, Cut Flowers, Vegetables, Beef Cattle, Grapes, Corn, Pasture & Rangeland, Hay

11 — Strawberries, Raspberries, Mushrooms, Lettuce, Bell Peppers, Nursery Plants, Orchids, Grapes

12 — Milk, Almonds, Tomatoes, Cherries, Beef Cattle, Poultry, Asparagus, Walnuts, Hay

13 — Timber, Beef Cattle, Pasture & Rangeland, Sheep, Poultry, Firewood

14 — Beef Cattle, Hay, Carrots, Turf, Sheep, Pasture & Rangeland

15 — Milk, Almonds, Grapes, Pistachios, Poultry, Cotton, Tomatoes, Onions, Oranges, Peaches

16 — Lettuce, Grapes, Strawberries, Broccoli, Cauliflower, Nursery Plants, Celery, Spinach

17 — Milk, Cotton, Almonds, Oranges, Pistachios, Carrots, Grapes, Beef Cattle, Hay

18 — Strawberries, Nursery Plants—Ornamental Trees & Bedding Plants, Avocados, Lemons, Vegetables

19 — Milk, Eggs, Nursery Plants, Beef Cattle, Grapes, Avocados, Grapefruit, Bell Peppers, Dates

20 — Nursery Plants—Indoor Foliage, Wood Ornamentals & Bedding Plants, Beef Cattle, Lettuce, Avocados, Alfalfa Hay, Tomatoes, Cut Flowers, Carrots, Broccoli

This map is intended to provide general 2004 agricultural crop information and county similarities. Information is based on County Agricultural Commissioner Reports.
1/09

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Figure 2.3. Various crops grown throughout California by region, including many of the crops DCNA is approved for use on throughout the state [31].

Pesticide Use Reports (PUR) from the California Department of Pesticide Regulation report many coastal counties in California as areas applying DCNA to crops such as celery, head lettuce, and leaf lettuce [32]. DCNA application, by pounds, in counties such as Monterey, Santa Clara, San Benito, and Ventura are shown in Figures 2.4-2.7. San Luis Obispo, Santa Barbara, Los Angeles, Orange, Santa Cruz, San Mateo, Kern, Fresno, Riverside, and Solano Counties also apply DCNA within their county borders. While DCNA is commonly applied to celery, head lettuce, and leaf lettuce within the coastal counties, it also is used on grapes in some of the same areas; for example, in 2010 DCNA was applied to grapes in Monterey County [32].
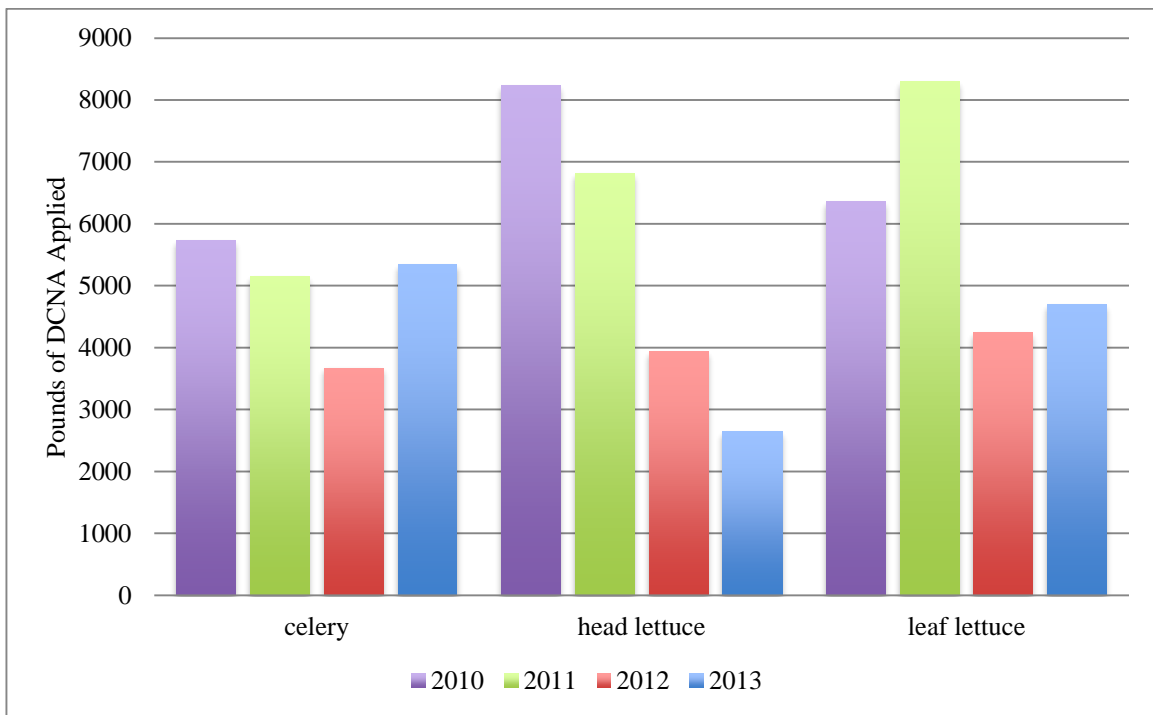
Figure 2.4. The amount of DCNA applied to celery, head lettuce, and leaf lettuce crops (in pounds) in Monterey County, CA from 2010-2013 [32].
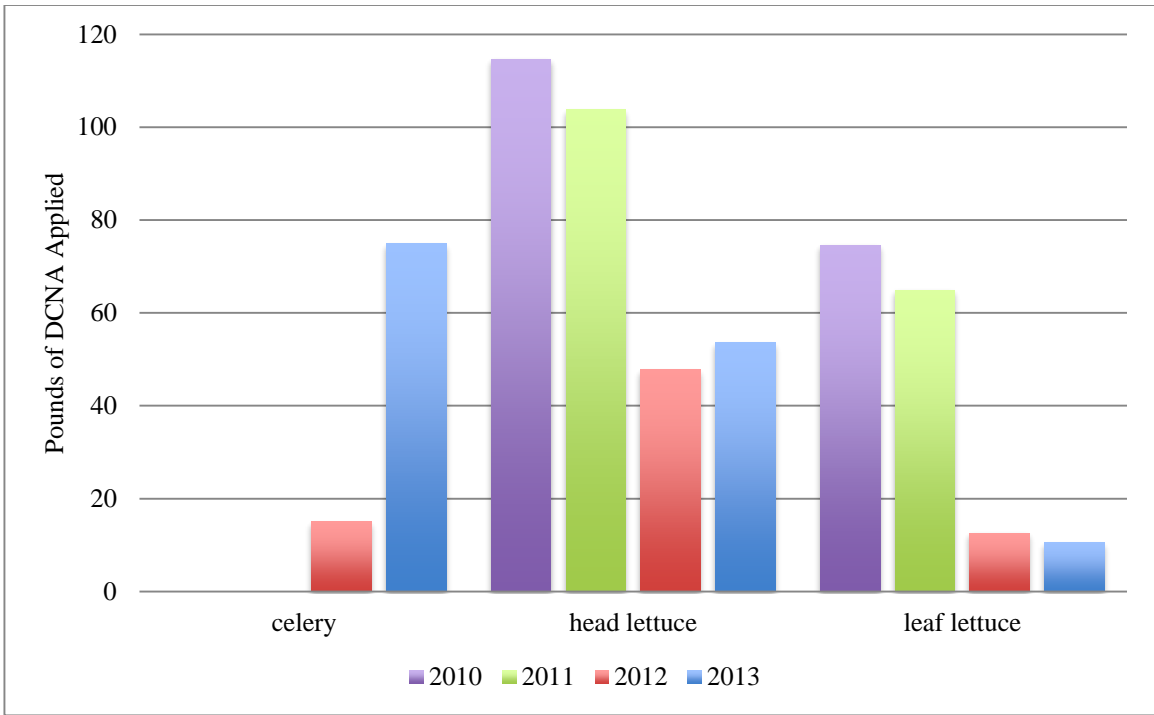
Figure 2.5. The amount of DCNA applied to celery, head lettuce, and leaf lettuce crops (in pounds) in Santa Clara County, CA from 2010-2013 [32].
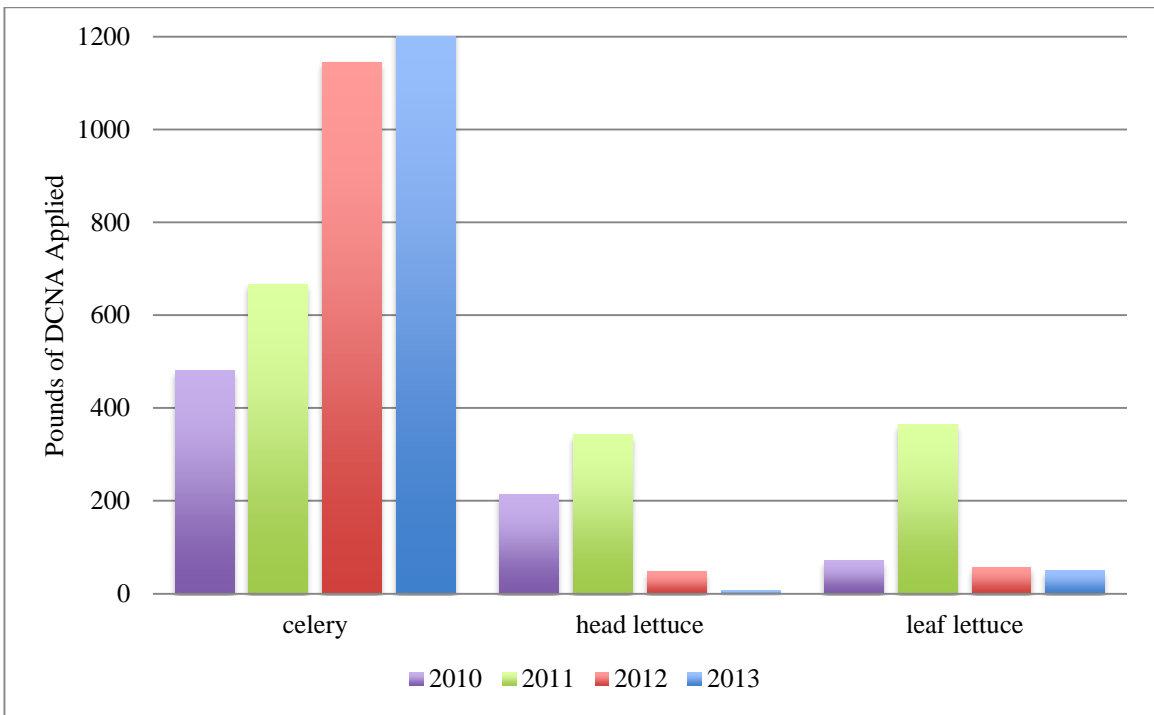
Figure 2.6. The amount of DCNA applied to celery, head lettuce, and leaf lettuce crops (in pounds) in San Benito County, CA from 2010-2013 [32].
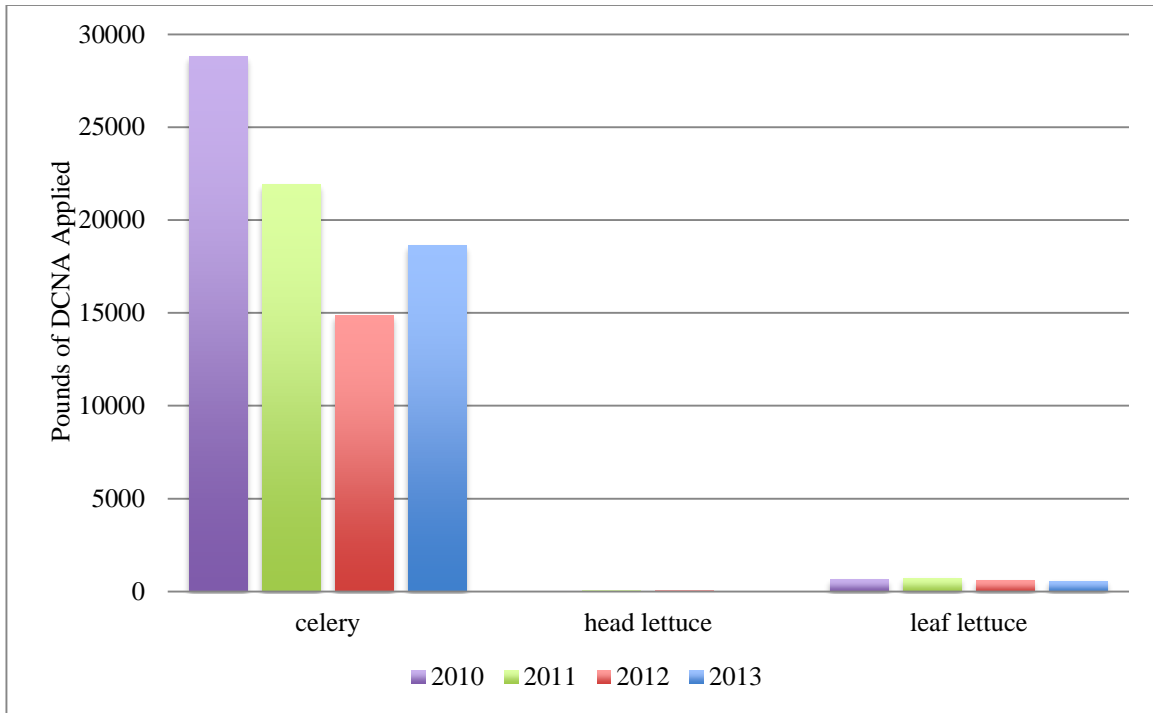
Figure 2.7. The amount of DCNA applied to celery, head lettuce, and leaf lettuce crops (in pounds) in Ventura County, CA from 2010-2013. Less than 40lbs was used annually on head lettuce [32].

The fate of DCNA in the environment is dependent on where it is applied and what type of waterbody the fungicide enters. It can contaminate surface water during application or enter surface water due to runoff [27]. DCNA is highly toxic to freshwater fish, while it is moderately toxic to freshwater invertebrates based on acute exposure studies [1]. No information was available on the acute or chronic toxicity information on marine or estuarine fish or invertebrates or chronic toxicity information on freshwater fish or invertebrates.

CHAPTER 3: MATERIALS AND METHODS

3.1. Chemical Reagents and Materials

Analytical grade 2,6-dichloro-4-nitroaniline (DCNA) and 2,6-dibromo-4-nitroaniline (DBNA) were obtained from Aldrich Chemical Company (St. Louis, MO). Sodium nitrate, sodium nitrite, sodium chloride, sodium bromide, maleic acid disodium salt hydrate, succinic acid disodium salt, and oxalic acid were used as standards and obtained also from Aldrich; fumaric acid was obtained from Fluka (St. Louis, MO). Potassium phosphate dibasic (anhydrous) was obtained from Mallinckrodt Chemical Company (Paric, KY). All solvents used were high performance liquid chromatography grade (HPLC); water was obtained from J. T. Baker (Center Valley, PA) and acetonitrile was obtained from Fisher Scientific (Pittsburgh, PA). Hydrochloric acid and sodium hydroxide solution were both obtained from Fisher Scientific (Fair Lawn, NJ). Instant Ocean® (Blacksburg, VA) was used to simulate seawater and mixed according to directions on the package. Natural estuarine water used for photolysis experiments was obtained from Lake Pontchartrain at Fontainebleau State Park in Mandeville, LA. Field and lab water samples were filter-sterilized prior to use using 0.22-micron cellulose acetate membrane filters obtained from Advantec MFS, Inc. (Dublin, CA).   Borosilicate glass 2 mL vials, clear vials for the photodegradation experiments and amber vials for dark controls, were obtained from Agilent Technologies (Santa Clara, CA).

3.2. Rate Experiments

DCNA and DBNA were dissolved in acetonitrile as a 1000 ppm stock solution. A 1.0 ppm solution for rate experiments was made from the stock solution, using various media including distilled water, 32 ppt artificial seawater, pH 7 0.01 M phosphate buffer in distilled water, and pH 7.6 8 ppt filter-sterilized estuarine water from Lake Pontchartrain. This value was

below the reported solubility of 6.3 mg/L, and the solvent was less than 1% of the total volume [31]. Vials containing 1.0 mL of the 1.0 ppm solution were irradiated in a SUNTEST XXL+ photochamber and were removed at regular intervals for analysis (t = 0, 2, 4, 6, 12, and 24 hours). Dark controls of DCNA and DBNA were run simultaneously. Residual DCNA and DBNA in solution were measured using an Agilent 1260 Infinity High Performance Liquid Chromatograph. DCNA and DBNA in solution were analyzed with photodiode array detection at 380 nm using a water and acetonitrile gradient mobile phase and a ZORBAX C-18 Eclipse Plus Analytical 4.6x150 mm 5-micron column.

The SUNTEST XXL+ photochamber irradiance was set at an irradiance control of 300-400 nm with a daylight filter system over a 24 hour time period. The phase length was 60 minutes with an energy output of 65 W/m^2. The temperature was set to 20°C, and the relative humidity was set to 20%. Each hour of exposure is equivalent to approximately 1.8 hours of exposure to June summer solstice sunlight at 30°N latitude [33].

The photodegradation of DBNA was measured to determine if halogens other then chlorine would impact the rate of degradation. Photonucleophilic substitution has been previously reported for compounds containing chlorine; using a similar compound that contains bromine instead of chlorine can help to determine if halogens follow the same or similar photonucleophilic pathways and processes. DCNA and DBNA were both irradiated in the photochamber and analyzed for residual product by HPLC.

3.3.   Degradation Products

HPLC analysis using analytical conditions identical to that of the parent compounds was used to plot the formation and decline of the major photoproducts resulting from the degradation

of DCNA. The photoproducts were measured using the same analysis conditions as the parent compounds but measured at 254 nm rather than 380 nm.

A Thermo Dionex ICS-5000+ Ion Chromatograph was used to measure the formation and decline of chlorine, bromine, nitrate, and nitrite ions. Analytes were separated on an AS-20 column using an isocratic mobile phase of 95:5 water and sodium hydroxide (50% w/w) at a 0.30 mL/min flow rate. Standards of the ions were run along with the DCNA and DBNA samples.

A ZORBAX SB-Aq Rapid Resolution HT 4.6x150 mm 1.8-micron column was used on the same HPLC as the parent compounds for the detection of the small aliphatic acids. Standards of succinic acid, maleic acid, fumaric acid, and oxalic acid were run prior to the irradiated samples. The mobile phase for detecting the acids was 99:1 isocratic 20 mM pH 2.0 phosphate buffer and acetonitrile with a 1.0 mL/min flow rate at 210 nm [34].

3.4. Data Analysis

At each time point (t = 0, 2, 4, 6, 12, 24 hours), the percent residual DCNA remaining in solution in vials were averaged and the pseudo-first order rate constant was calculated from a plot of the natural logarithm of the percent remaining (ln C/Co) over time, where ln C/Co = -kt, where k = the pseudo-first order rate constant in hr^{-1}. The half-life was calculated from the rate constant where $t_{1/2}$ = ln 2/k. One-way ANOVA was used for statistical analysis of the degradation rates and half-lives, using α = 0.05. Both Proc. Mixed LS Means and Diff. LS Means options in ANOVA were used. Each trial was done in triplicate, with the exception of DCNA in artificial seawater, DBNA in distilled water, and DBNA in artificial seawater.

CHAPTER 4: RESULTS AND DISCUSSION

4.1.   Degradation Rate and Half-Life

Both DCNA and DBNA samples were rapidly degraded in all media by simulated

sunlight. No degradation was observed in any dark control sample. The degradation rate

constants and half-lives are shown in Table 4.1. DCNA degrades quickly, with a calculated half-

life of 7.62 ± 0.094, 7.37 ± 0.279, 5.78 ± 0.216, and 6.29 ± 0.048 hours in distilled water,

artificial seawater, phosphate buffer, and estuarine water respectively. DBNA degraded at a

slightly slower rate than DCNA with calculated half-lives of 8.86 ± 0.257, 8.31 ± 0.144, $8.20 \pm$

0.059, and 7.56 ± 0.181 hours in distilled water, artificial seawater, phosphate buffer, and

estuarine water respectively.

Table 4.1. The calculated rate constants and half-lives of both DCNA and DBNA. The degradation of the samples was measured in distilled water, artificial seawater, phosphate buffer, and estuarine water, as determined from HPLC analysis.

	Rate Constant (hr^{-1})	Half-Life (hours)	Samples (n)
DCNA			
Distilled Water	0.092 ± 0.0011	7.62 ± 0.094	3
32 g/L Artificial Seawater	0.094 ± 0.0036	7.37 ± 0.279	7
pH 7 0.01M Phosphate Buffer	0.120 ± 0.0046	5.78 ± 0.216	3
Filter-Sterilized Estuarine Water	0.110 ± 0.0009	6.29 ± 0.048	3
DBNA			
Distilled Water	0.078 ± 0.0023	8.86 ± 0.257	4
32 g/L Artificial Seawater	0.083 ± 0.0014	8.31 ± 0.144	4
pH 7 0.01M Phosphate Buffer	0.085 ± 0.0006	8.20 ± 0.059	3
Filter-Sterilized Estuarine Water	0.091 ± 0.0021	7.56 ± 0.181	3
* No degradation observed in dark controls.			

One-way ANOVA analysis showed a slight significant difference in degradation rate for

DCNA in phosphate buffer and estuarine water then in artificial seawater or DI water, however

these differences may be artificial as rates could have been skewed by the latest time points as

there was no significant difference between rates through the first half-life in any case. The trends in the degradation rates for DCNA and DBNA are shown in Figures 4.1 and 4.2 for each of the four media. DBNA degradation behaved similar to DCNA; no significant differences were observed for rates in any media except estuarine water and those were only slightly significant. In both cases it is possible that indirect photolysis processes could have been responsible for some of the observed differences in estuarine water from the other media however these would appear to be minor to direct photolysis processes.

The half-life of DCNA was observed to be shorter than DBNA, despite the similarities in their structures. While they follow similar degradation trends, the difference in half-life suggests that the ring-halogen affects the rate of degradation. Chlorine is a stronger electron-withdrawing group, which could explain why DCNA degrades faster than DBNA. This impacts both the UV absorption spectrum, with DCNA having a slightly greater molar absorptivity within the sunlight region. This difference could explain the slight differences in the rate.

Both DCNA and DBNA seem to behave more similarly to 3,4-dichloroaniline (DCA) than pentachlorophenol (PCP) or 4-hydroxychlorothalonil; the photodegradation rate and half-life of both DCNA and DBNA were not impacted by the salinity of the lab controlled waters, distilled water and artificial seawater [2, 6]. Photonucleophilic substitution reactions involving the chlorine moieties do not appear to be affecting the rate as they did in compounds such as pentachlorophenol (PCP) and hydroxychlorothalonil, where the degradation rates showed significant difference between distilled water and artificial seawater [2, 5, 13]. It is possible that the initial rate of degradation is dominated by the initial loss of nitrate rather then the initial loss of chlorine.
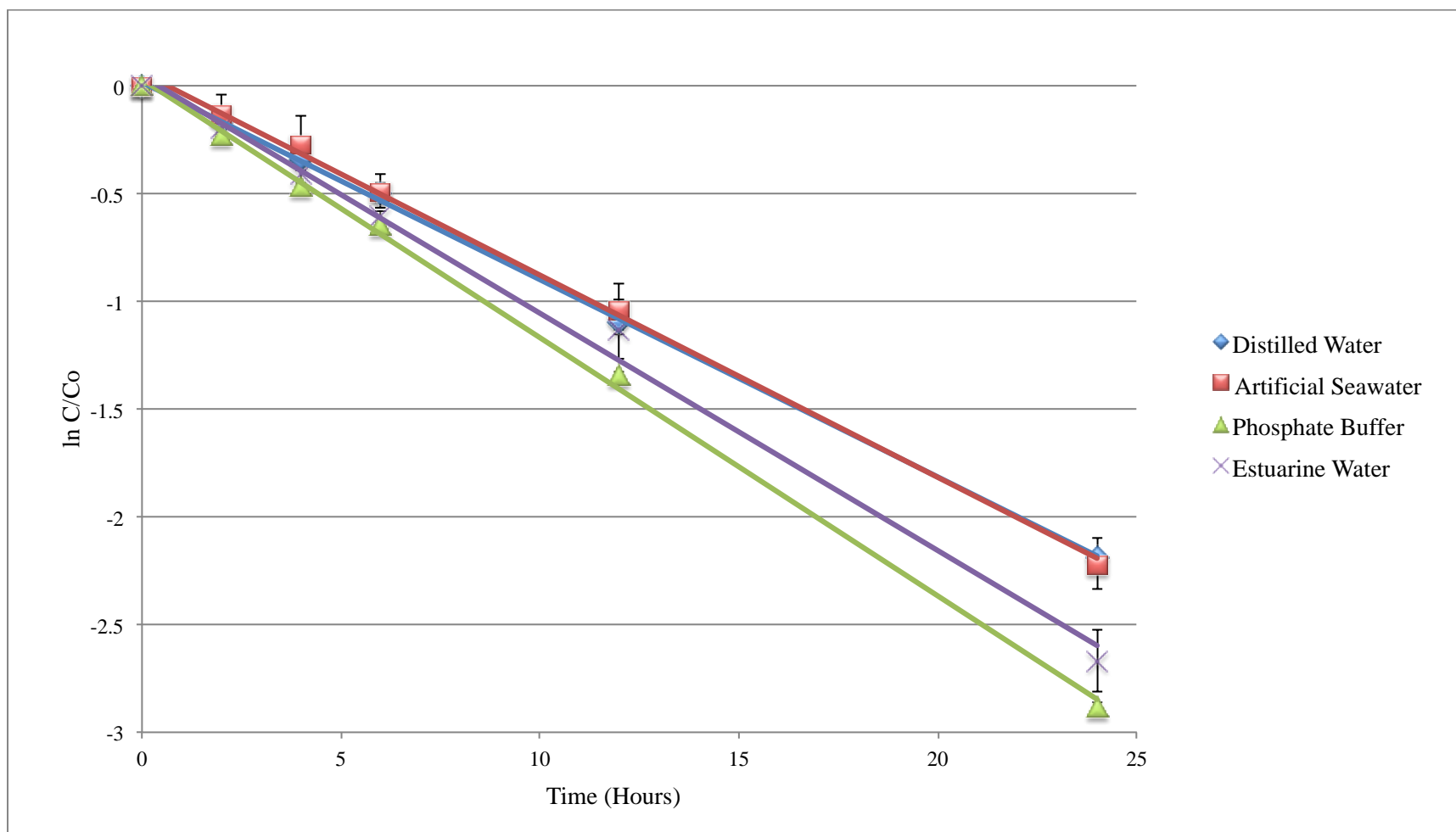
Figure 4.1. The degradation rates of DCNA in distilled water, artificial seawater, phosphate buffer, and estuarine water. While there is no significant difference within the first half-life, the difference is being skewed by the later data points in the following half-lives. Error bars represent the standard deviations of each data set.
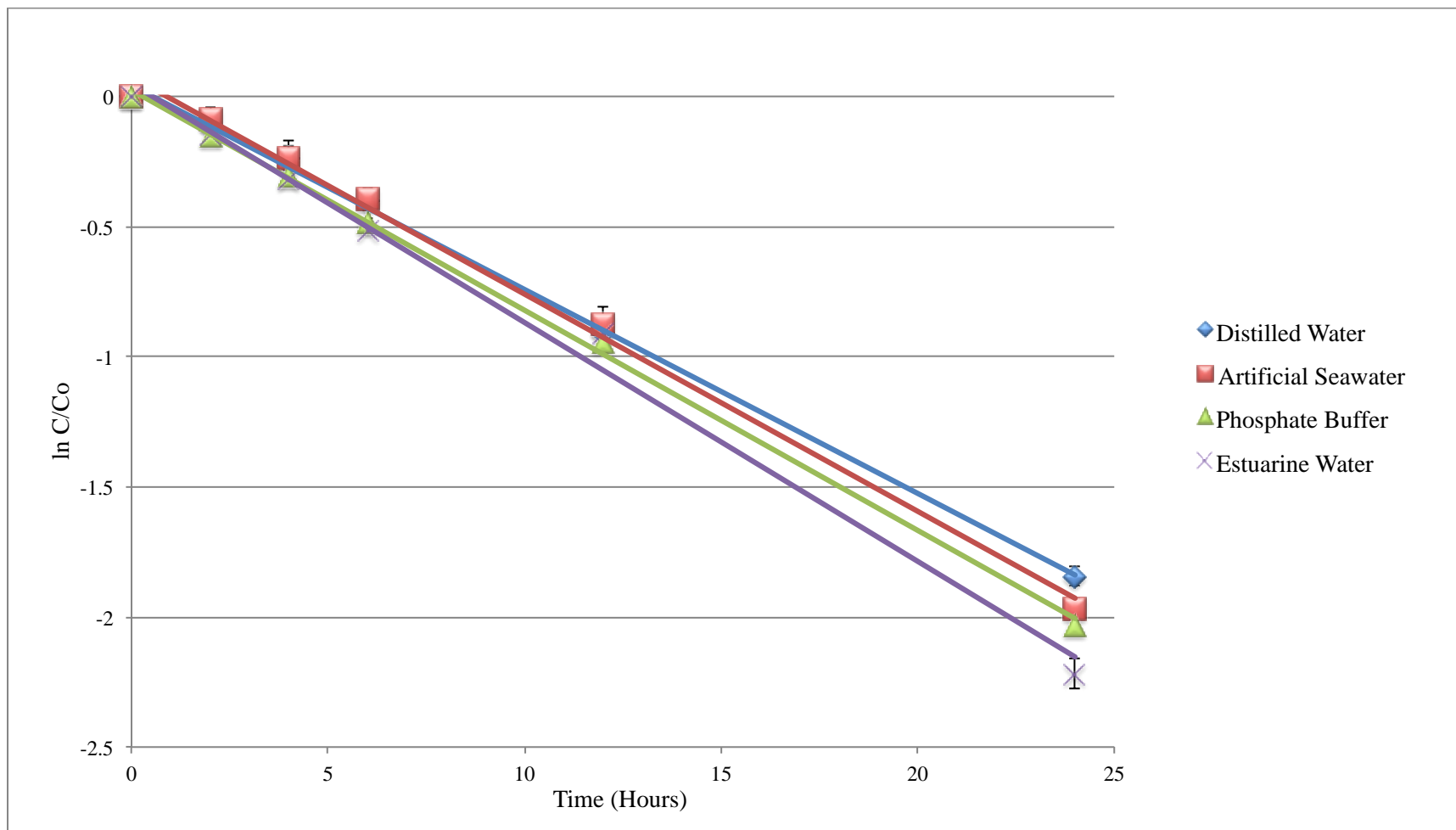
Figure 4.2. The degradation rates of DBNA in distilled water, artificial seawater, phosphate buffer, and estuarine water. While there is no significant difference within the first half-life, the difference is being skewed by the later data points in the following half-lives. Error bars represent the standard deviations of each data set.

Ion chromatography data showed the immediate generation of chlorine, nitrite, and nitrate ions that were all detected at the two-hour time point. Chlorine and nitrate continued to form over time while nitrite formed quickly and began to decline, presumably by photolysis to nitrate. While the quick formation of both nitrite and chlorine ions supports that photonucleophilic substitution may be occurring in the degradation of DCNA, other processes such as direct cleavage of the substituents from the aromatic ring to generate a nitrite and chloride radical cannot be ruled out. The formation of nitrate ions is generally delayed, compared to nitrite and chlorine. Nitrate is likely generated as the result of direct photolysis of nitrite ions [18, 19]. Nitrate ions appear to be increasing as irradiation continues, while the amount of nitrite detected appears to be decreasing. The formation and degradation of chlorine, bromine, nitrite, and nitrate ions for DCNA and DBNA in distilled water is shown in Figures 4.3 and 4.4.

Chlorine is a stronger electron-withdrawing group than bromine, which may explain why more chlorine ions were detected by ion chromatography than bromine ions and also support why DCNA has a shorter half-life than DBNA. Since chlorine is a stronger electron-withdrawing group, it would more greatly activate the ring to photonucleophilic substitution processes facilitating the loss of the nitro moiety. The rate of degradation does not seem to be driven solely by the loss of chloride from the molecule. The loss of nitrite from the structure appears to play an obvious and significant role. Both chlorine and nitro groups are subject to photonucleophilic substitution [2, 12].
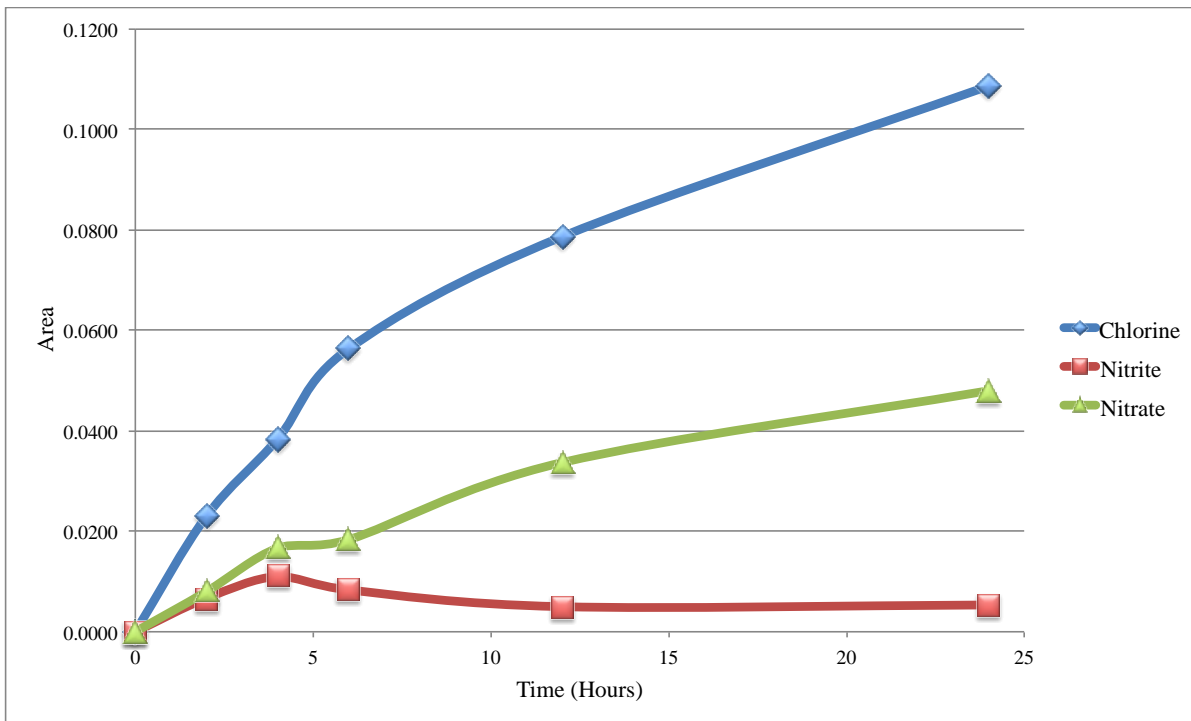
Figure 4.3. The generation of chlorine, nitrite, and nitrate ions from the degradation of DCNA in distilled water.
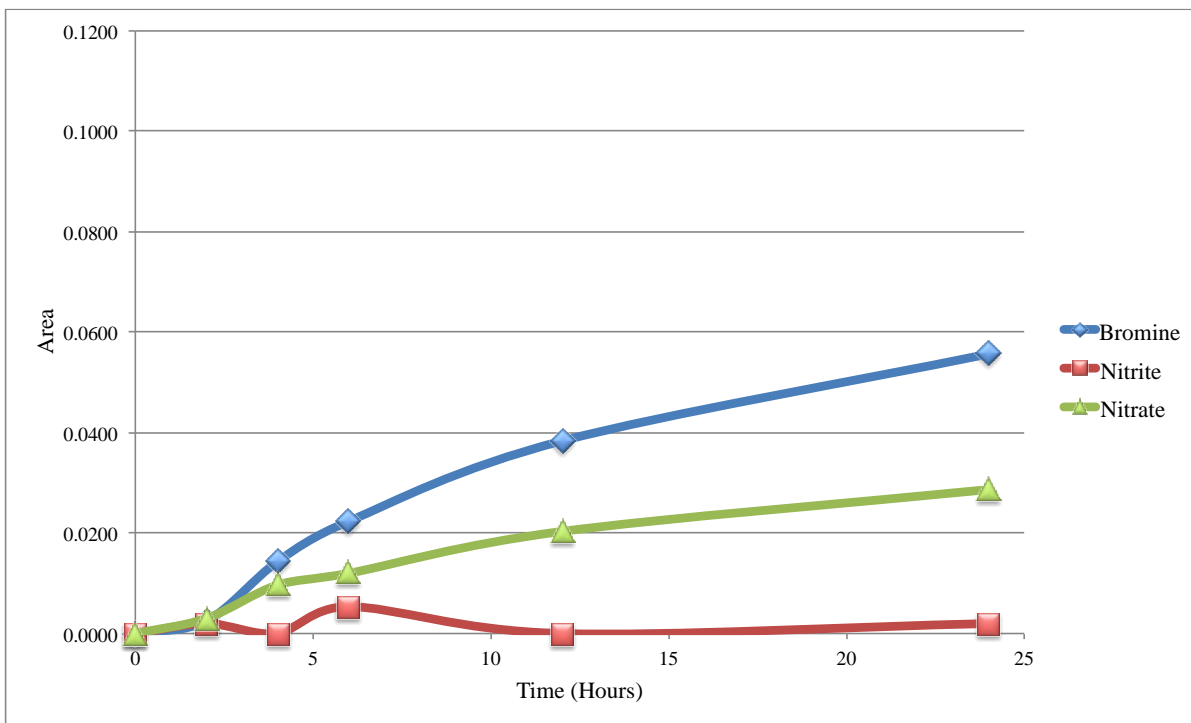
Figure 4.4. The generation of bromine, nitrite, and nitrate ions from the degradation of DBNA in distilled water.

4.2.   Degradation Products

      While seawater did not appear to impact the degradation of DCNA, significant

differences in formation and decline were observed in intermediate, unidentified degradation

products, for both DCNA and DBNA. Figures 4.5 and 4.6 show the formation and decline of the

unidentified, intermediate photoproducts of DCNA and DBNA in distilled water and artificial

seawater. In the case of DCNA, the peak observed at 5 minutes follows the same trends in both

seawater and distilled water; this appears to behave similarly as the peak at 6 minutes in DBNA.

This product appears to form the at nearly the same proportion and at the same rate in both

distilled water and artificial seawater, and also degrade at the same rate. However, the peak at 8

minutes in DCNA appears to be significantly affected by seawater, presumably the salinity. In

artificial seawater, the amount of the unidentified product at 8 minutes appears form at double

the amount than it forms in distilled water. By 12 hours of irradiation, much of the product has

been degraded in both distilled water and artificial seawater, and by 24 hours of irradiance the

product is no longer detected. Therefore, the amount of product formed appears to be affected

by salinity but the product appears to still degrade quickly in both media. The peak at 9 minutes

in DBNA samples appears to follow a similar trend to the peak at 8 minutes in DCNA.

      Similar to the generation of chlorine and bromine ions mentioned in Chapter 4.1, the

intermediate photoproducts of DCNA appear to form at higher relative amounts based upon peak

area than those of DBNA however this can only be confirmed after the identity of the products is

verified, since it is likely that different compounds will have different molar absorptivities and

respond differently to UV detection.
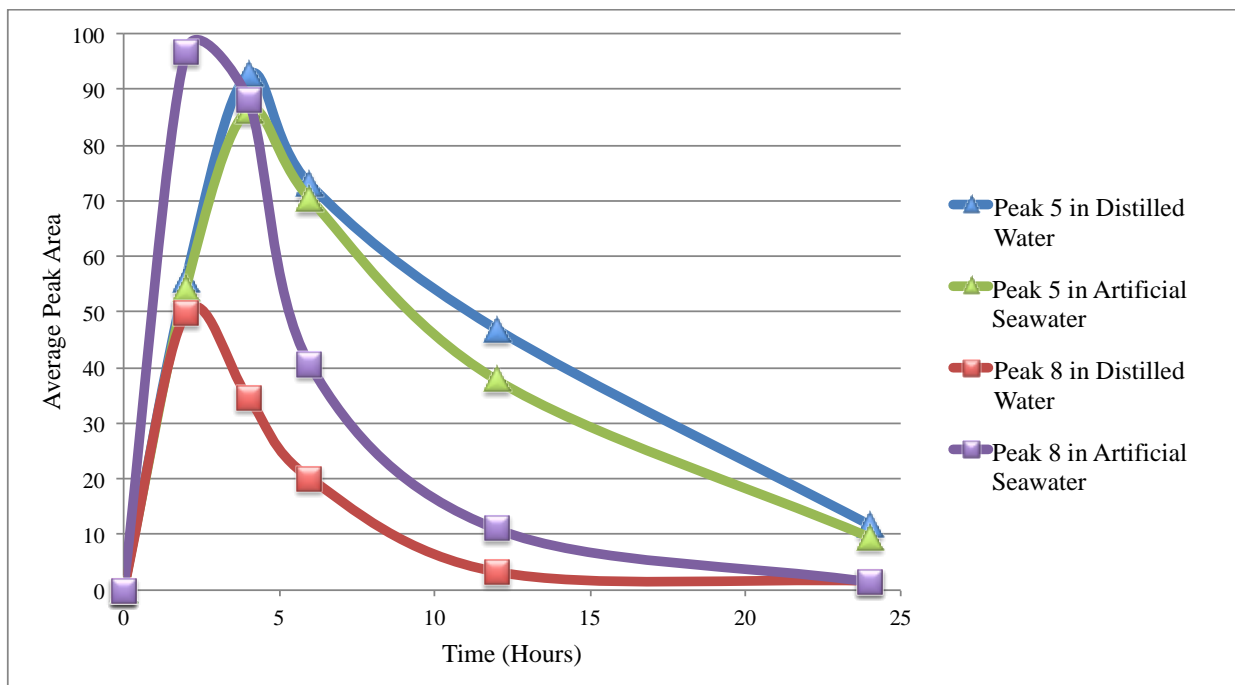
Figure 4.5. The unidentified intermediate photoproducts of the degradation of DCNA in distilled water and artificial seawater. Peak number refers to HPLC retention time.
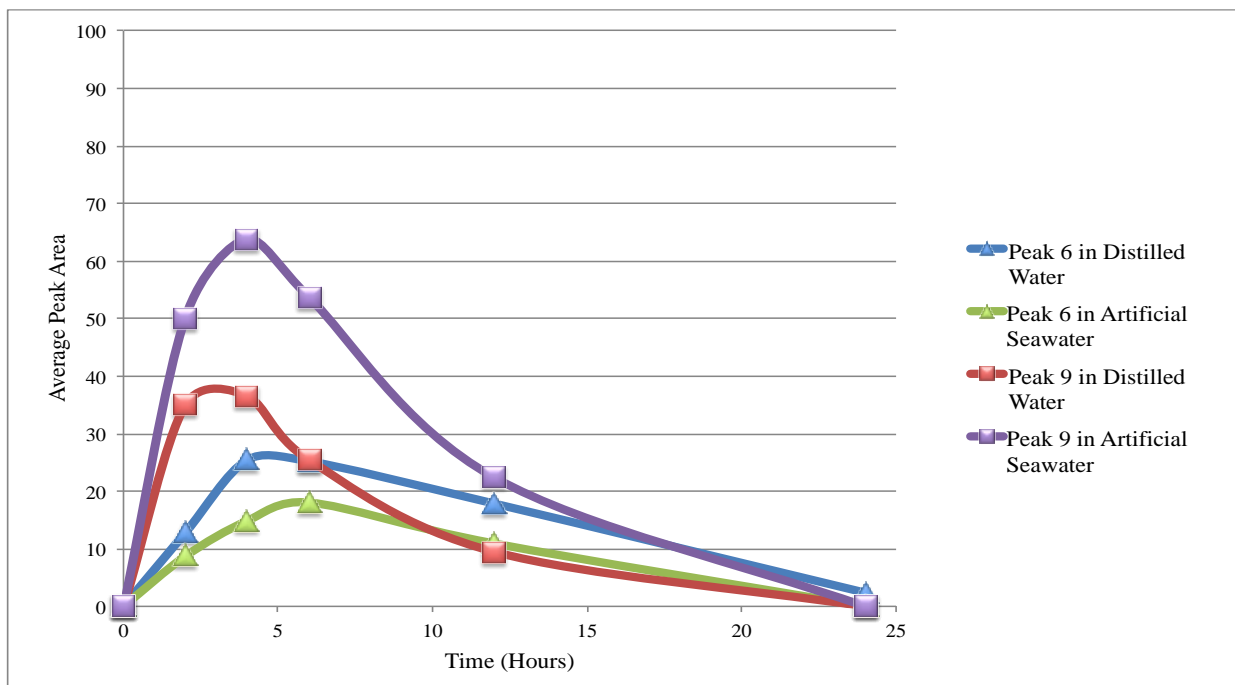
Figure 4.6. The unidentified intermediate photoproducts of the degradation of DBNA in distilled water and artificial seawater. Peak number refers to HPLC retention time.

While the degradation of the parent-compounds in distilled water and artificial seawater was similar to the degradation of 3,4-dichloroaniline (DCA), the formation and degradation of the intermediate products behaves similarly to pentachlorophenol (PCP). As mentioned in Chapter 2.4, the major photoproduct of PCP was identified to be tetrachlorophenol. In distilled water, 0.8% of the product was measured to be tetrachlorophenol while in artificial seawater 6.1% of the product was tetrachlorophenol. This also applies to DCA. The major photoproduct of DCA was 3-chloroaniline; 2.0% of this product was measured in distilled water and 5.0% was measured in artificial seawater [2]. It is possible that a similar process may be occurring also for DCNA and DBNA. This can only be confirmed through the identification of degradation products.

At 12 hours of irradiation, small amounts of the aliphatic acids maleic and fumaric acid were detected in DCNA samples. As observed in the photodegradation of PCP and hydroxychlorothalonil, the same small aliphatic acids appear to be the terminal degradation products of compounds likely to undergo photonucleophilic substitution [2, 13, 14]. The formation of these products late in the degradation pathway indicates that substituents are initially lost from the ring and that ultimately the aromatic ring itself if further degraded.

CHAPTER 5: CONCLUSIONS

The rate of degradation and half-life of DCNA does not appear to be affected by the salinity of water. When measured in distilled water, artificial seawater, phosphate buffer, and estuarine water, no significant difference in degradation was measured within the first half-life. The same trends are observed in the degradation of DBNA.

Immediate generation of both chlorine and nitrite ions, detected by ion chromatography, suggests that photonucleophilic processes may be occurring however other processes cannot be ruled out. The later generation of nitrate ions supports the further photolysis of nitrite ions forming nitrate. Hydroxyl radicals generated in this latter process may further contribute to the degradation of the parent compound and subsequent degradation products. Bromine ions were generated immediately as well, but not to the same degree as chlorine ions. This is possibly due to the fact that chlorine is a stronger electron-withdrawing group than bromine, as well as supporting that DCNA has a shorter calculated half-life than DBNA.

Two unidentified intermediate photoproducts were detected in the degradation of DCNA and DBNA analysis by HPLC. The peak at 5 minutes in DCNA samples does not appear to be affected by the salinity of the water, however the peak at 8 minutes does appear to be significantly affected by the salinity of the water. The 8-minute peak nearly doubles in concentration when irradiated in artificial seawater as compared to distilled water. DBNA follows similar trends with analogous photoproducts detected at 6 minutes and 9 minutes.

While the salinity of water does not appear to affect the overall rate of degradation of DCNA, it does affect the generation of the photoproducts.

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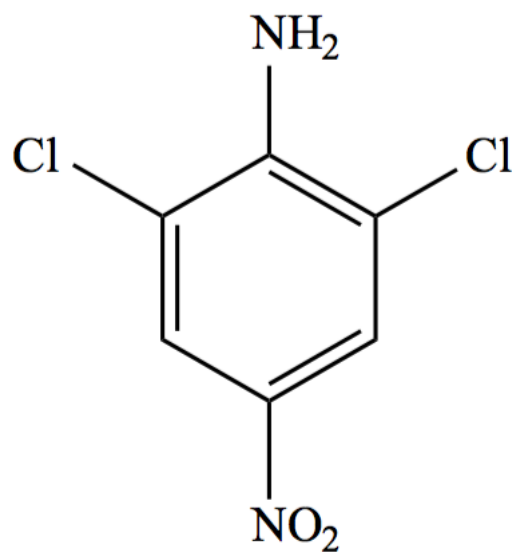
Figure A.1. The structure of 2,6-dichloro-4-nitroaniline (DCNA).
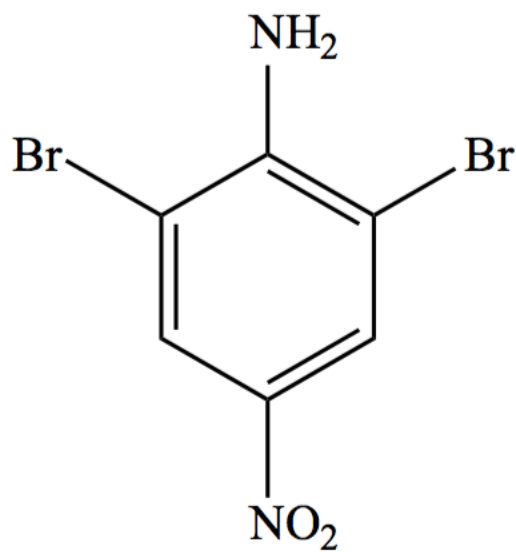
Figure A.2. The structure of 2,6-dibromo-4-nitroaniline (DBNA).
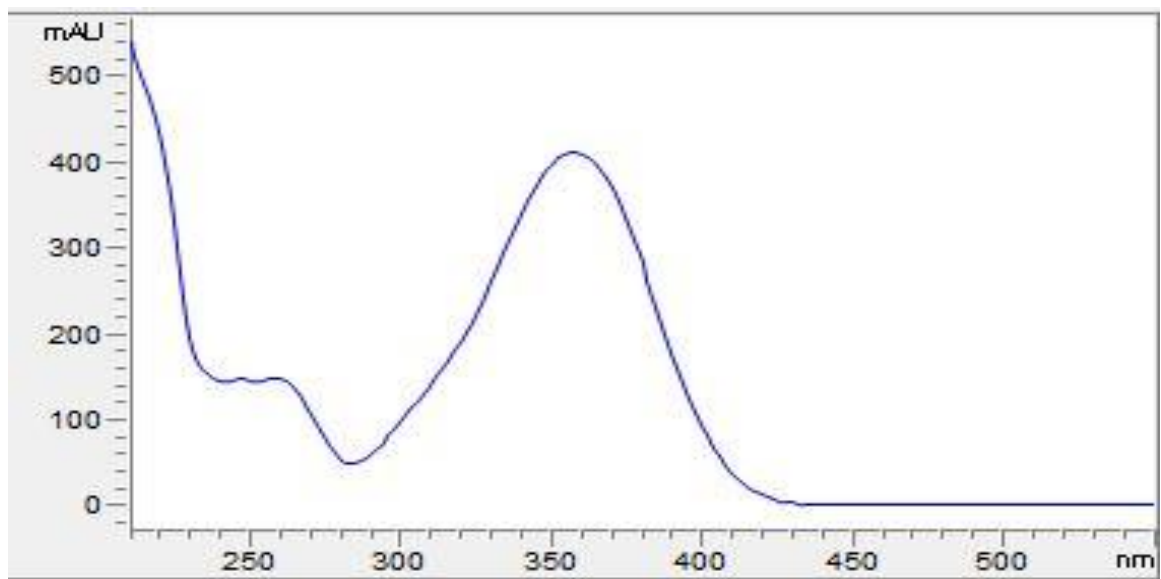
Figure A.3. UV spectrum for DCNA.
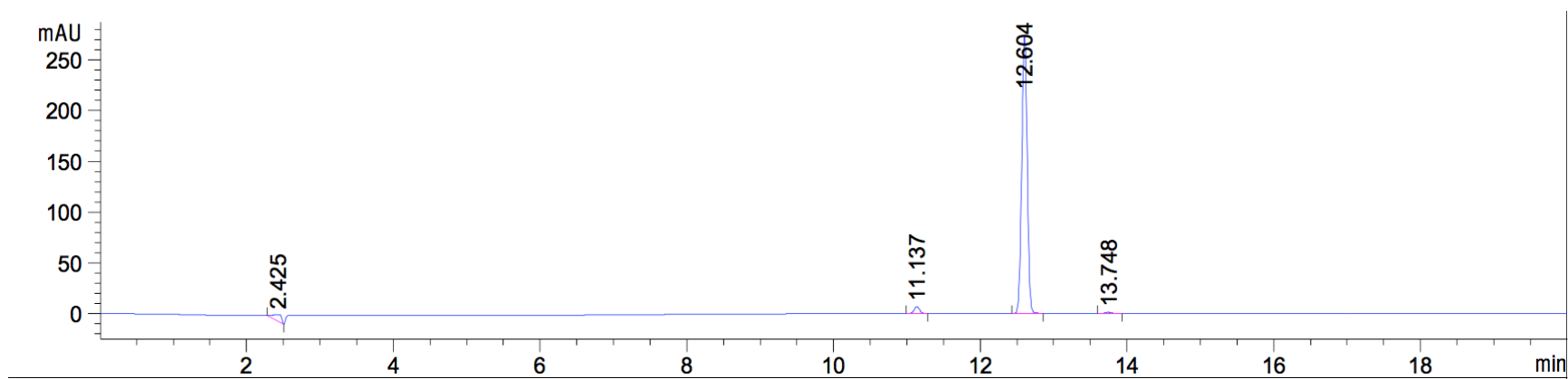
Figure A.4. HPLC spectrum for DCNA in distilled water at t=0 hours. 12.604 minutes at 380nm.
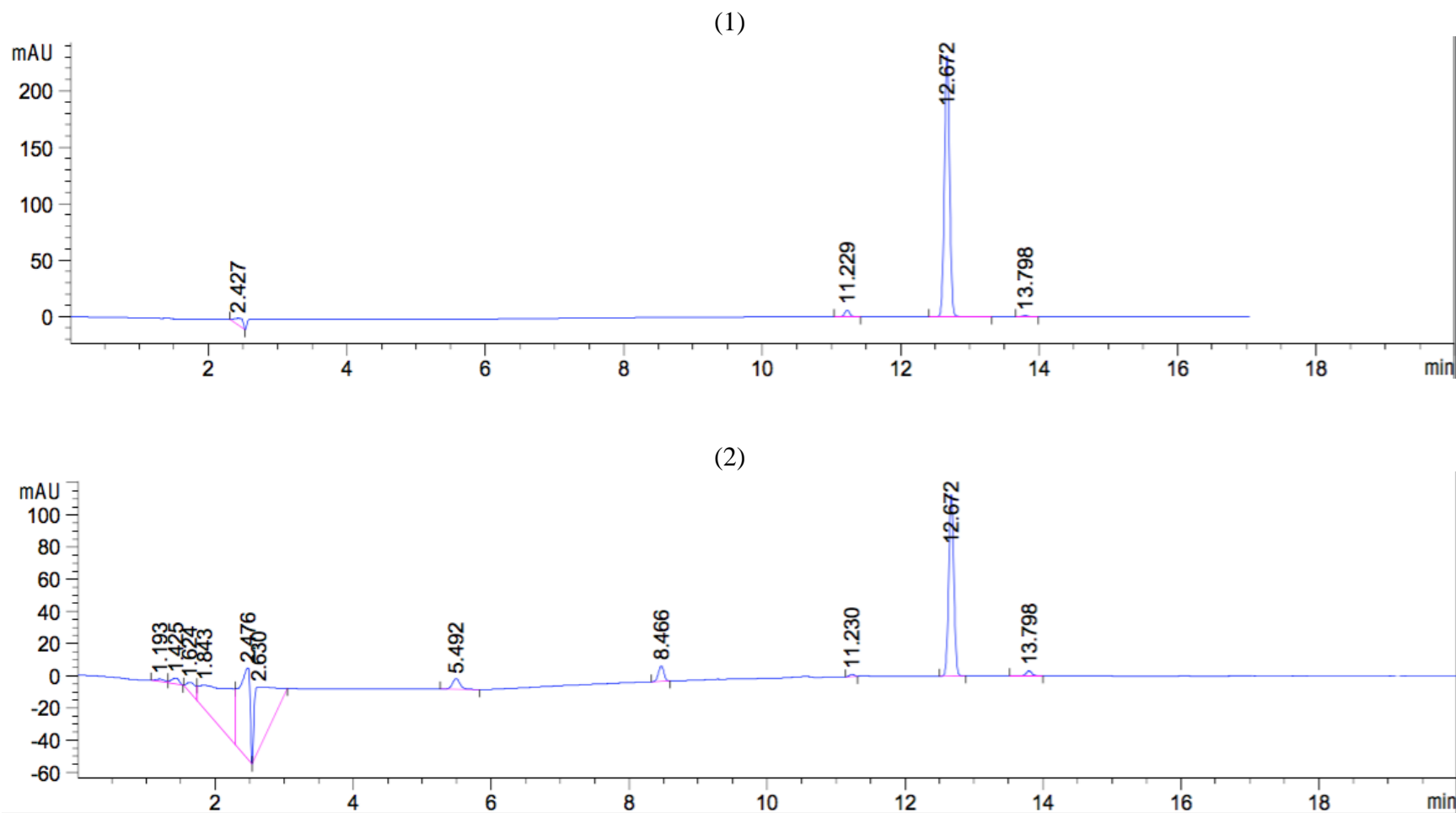
Figure A.5. HPLC spectra for DCNA at t=2 hours. (1) Shows the decrease in concentration of DCNA, retention time 12.672, at 380 nm. (2) Shows the formation of the photoproducts at 5.492 minutes and at 8.466 minutes at 254 nm.
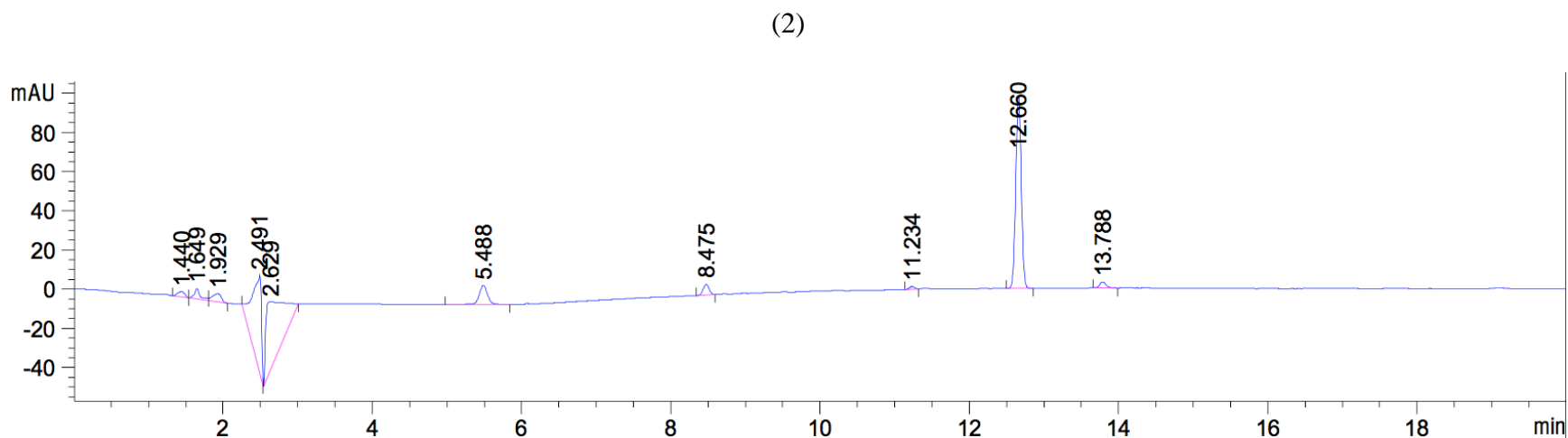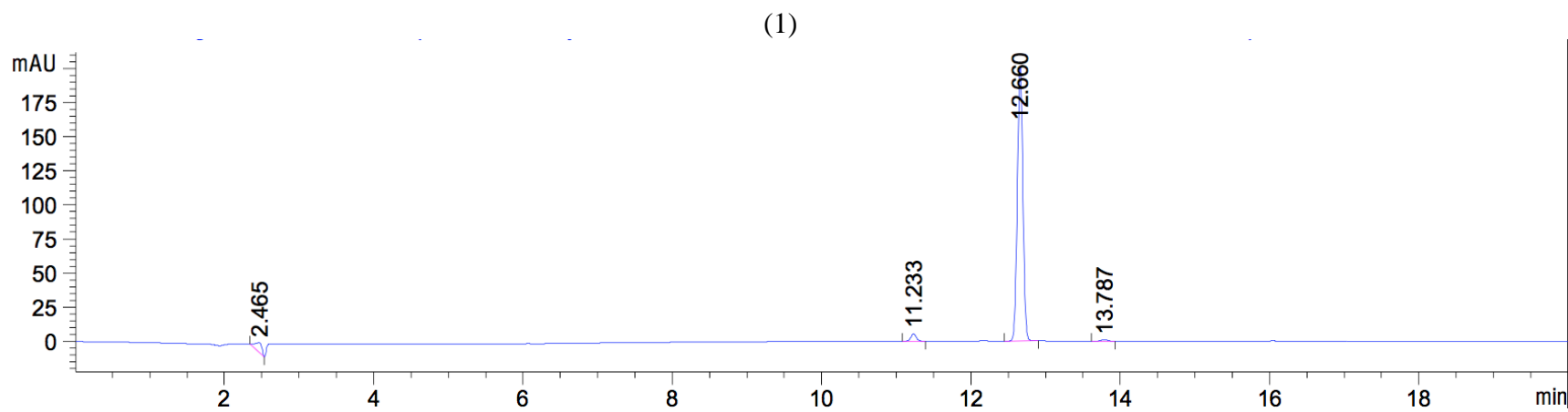
Figure A.6. HPLC spectra for DCNA at t=4 hours. (1) Shows the decrease in concentration of DCNA, retention time 12.660, at 380 nm. (2) Shows the formation of the photoproducts at 5.488 minutes and at 8.475 minutes at 254 nm.
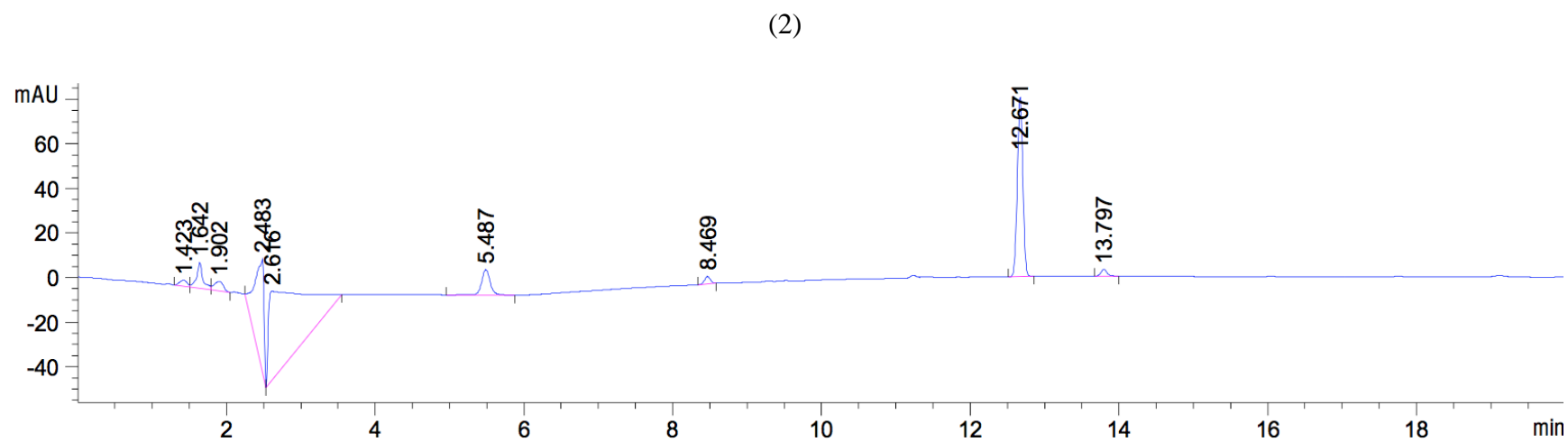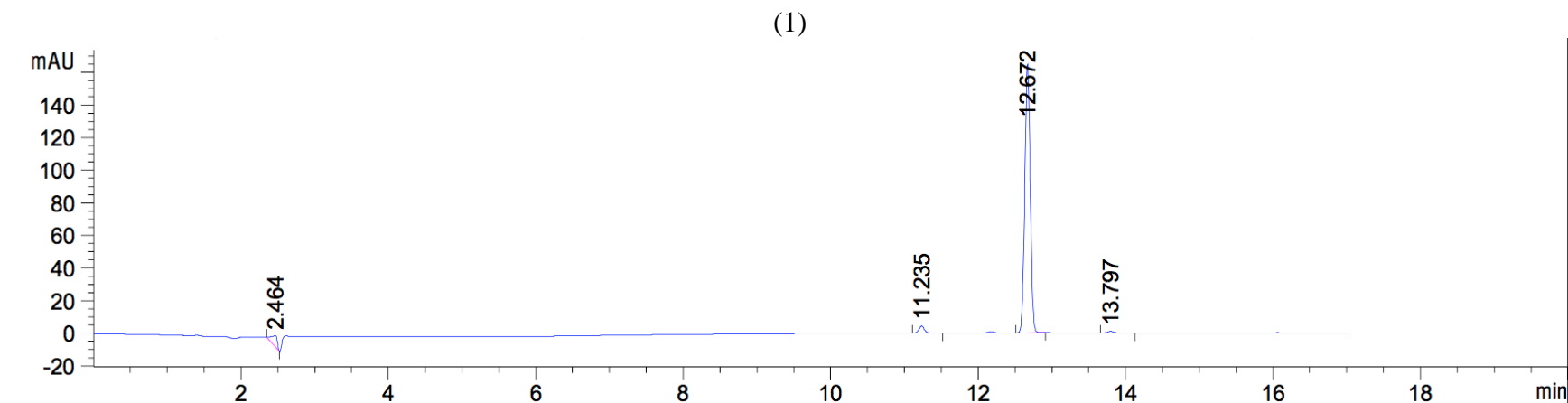
Figure A.7. HPLC spectra for DCNA at t=6 hours. (1) Shows the decrease in concentration of DCNA, retention time 12.672, at 380 nm. (2) Shows the formation of the photoproducts at 5.487 minutes and at 8.469 minutes at 254 nm.
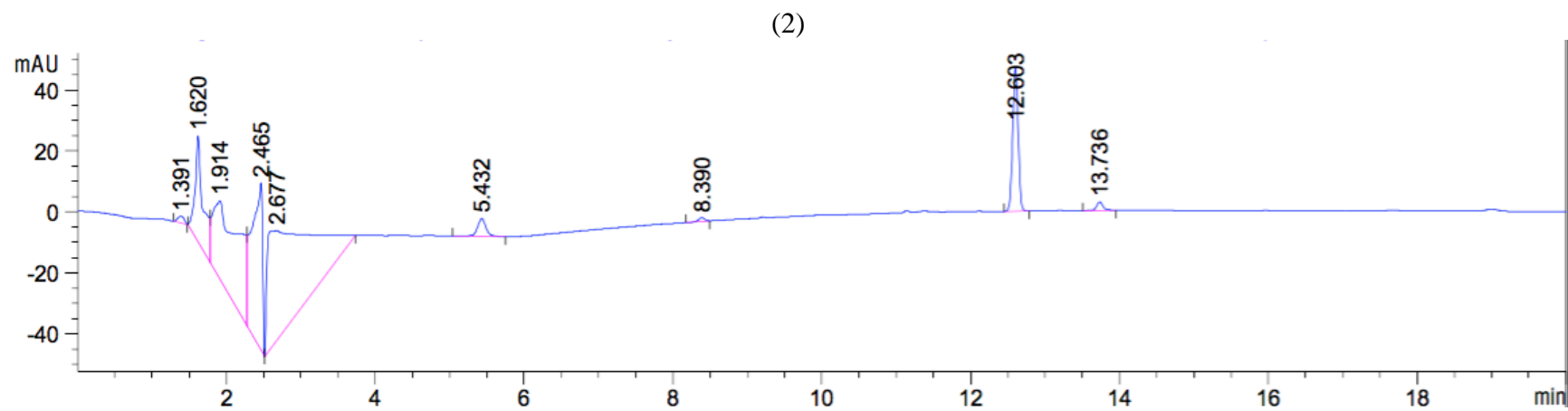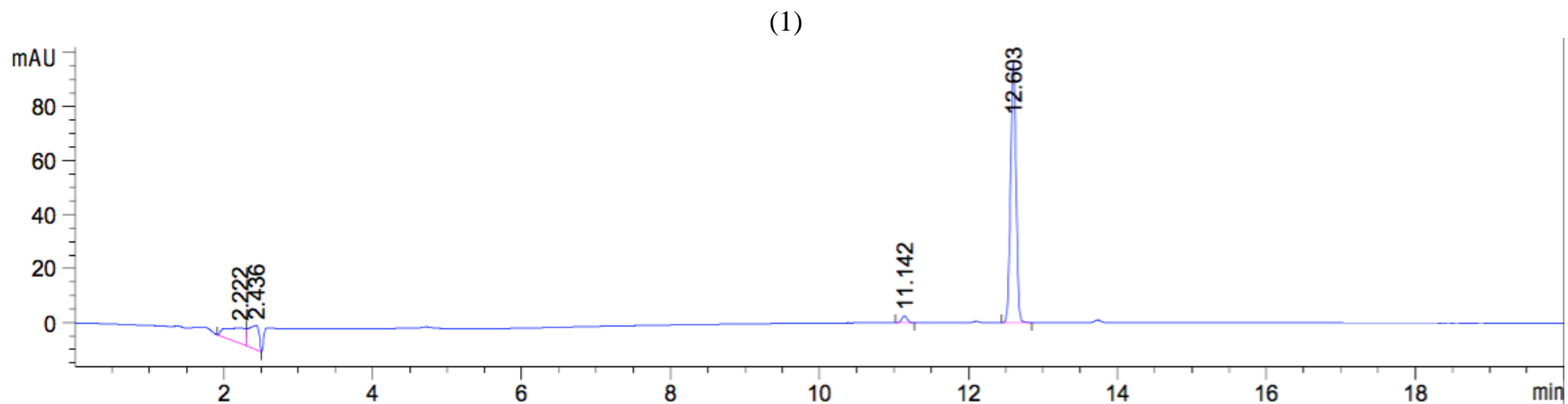
Figure A.9. HPLC spectra of DCNA at t=12 hours. (1) Shows the decrease in concentration of DCNA, retention time 12.603, at 380 nm. (2) Shows the degradation of the photoproducts at 5.432 minutes and at 8.390 minutes at 254 nm.
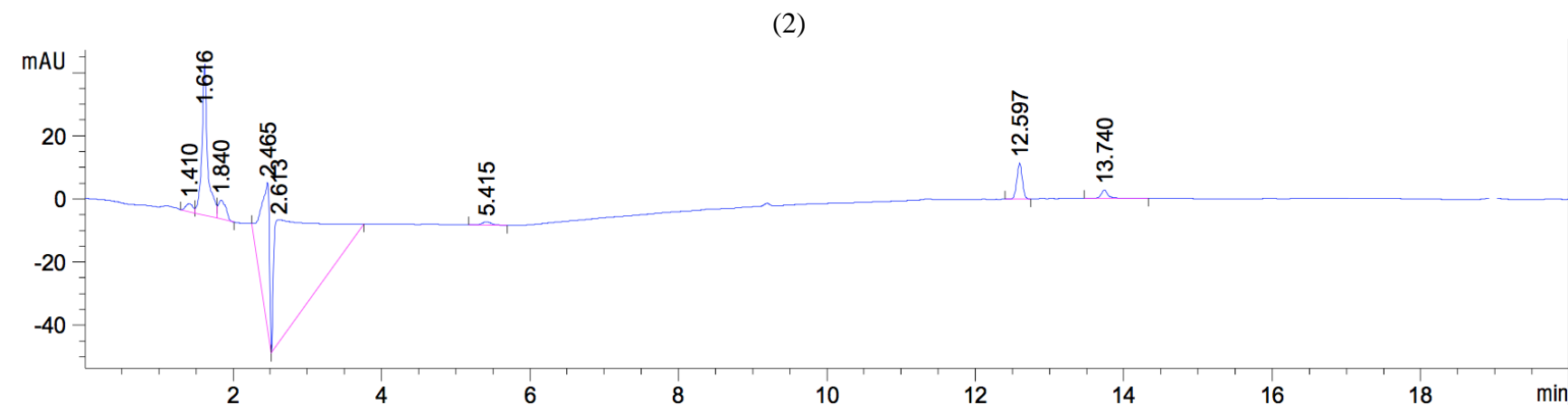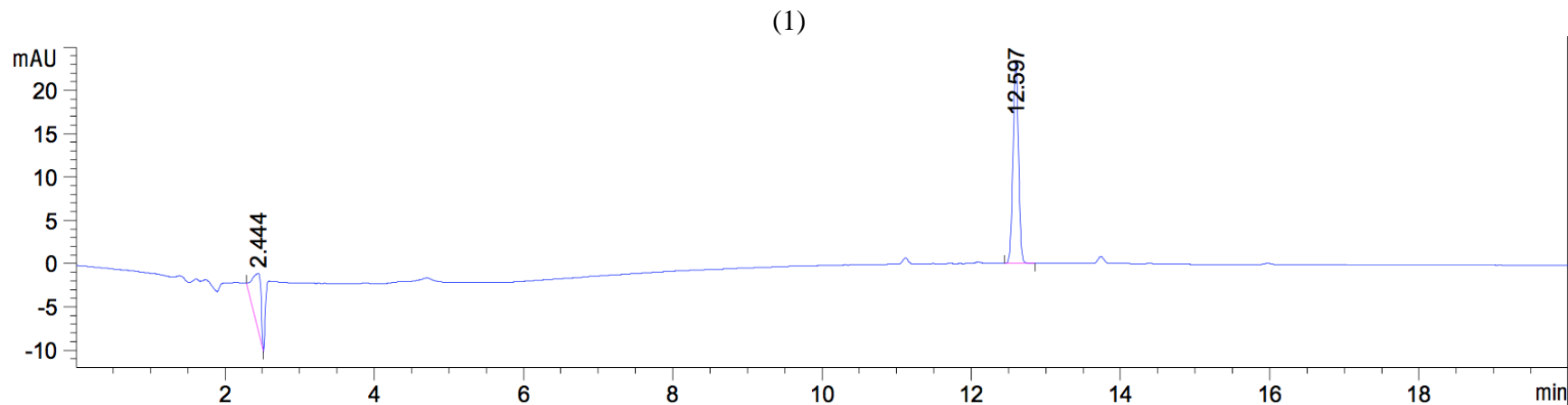
Figure A.10. HPLC spectra for DCNA at t=24 hours. (1) Shows the decrease in concentration of DCNA, retention time 12.660, at 380 nm after 24 hours of irradiation. (2) Shows the degradation of the photoproduct at 5.415 minutes and that the other product is no longer measurable after 24 hours of constant irradiation, measured at 254nm.
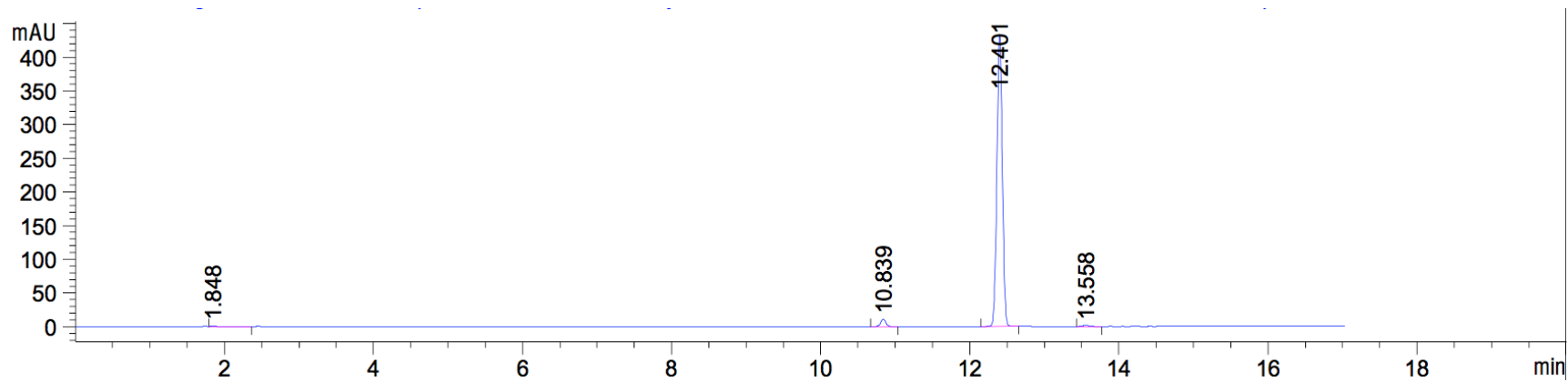
Figure A.11. HPLC spectrum for DCNA at t=0 in artificial seawater at 380nm.
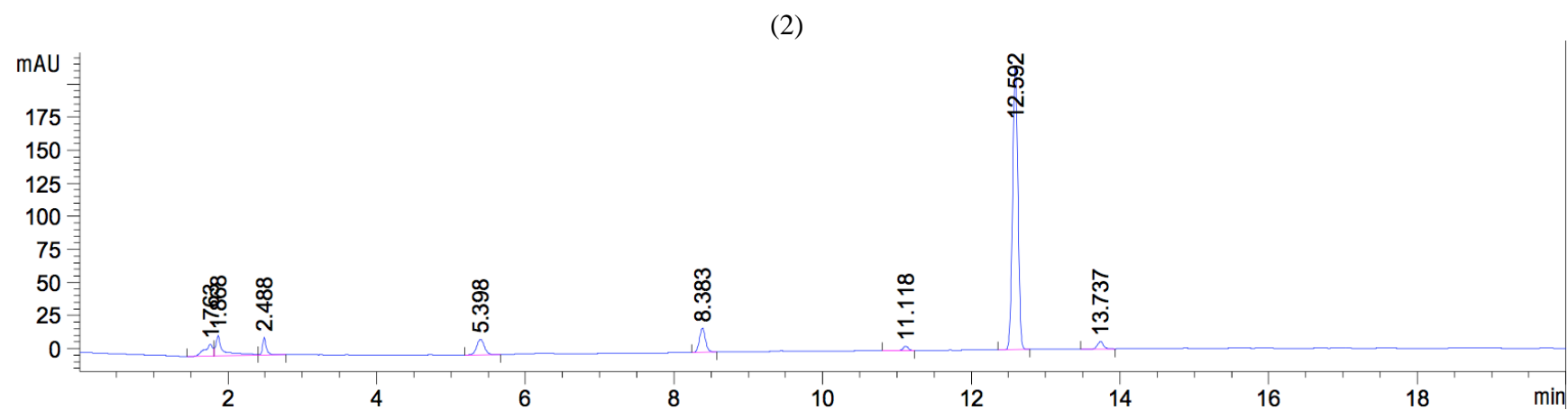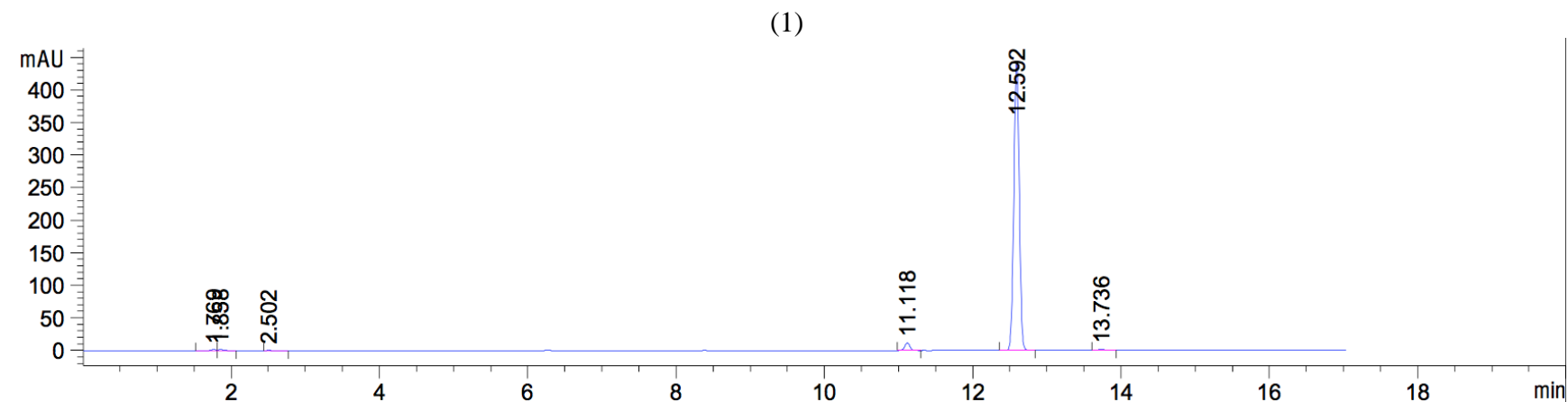
Figure A.12. HPLC spectra of DCNA at t=2 hours in artificial seawater. (1) DCNA degradation at 2 hours of irradiation measured at 380nm. (2) The formation of the products at 5.398 minutes and at 8.383 minutes measured at 254nm.
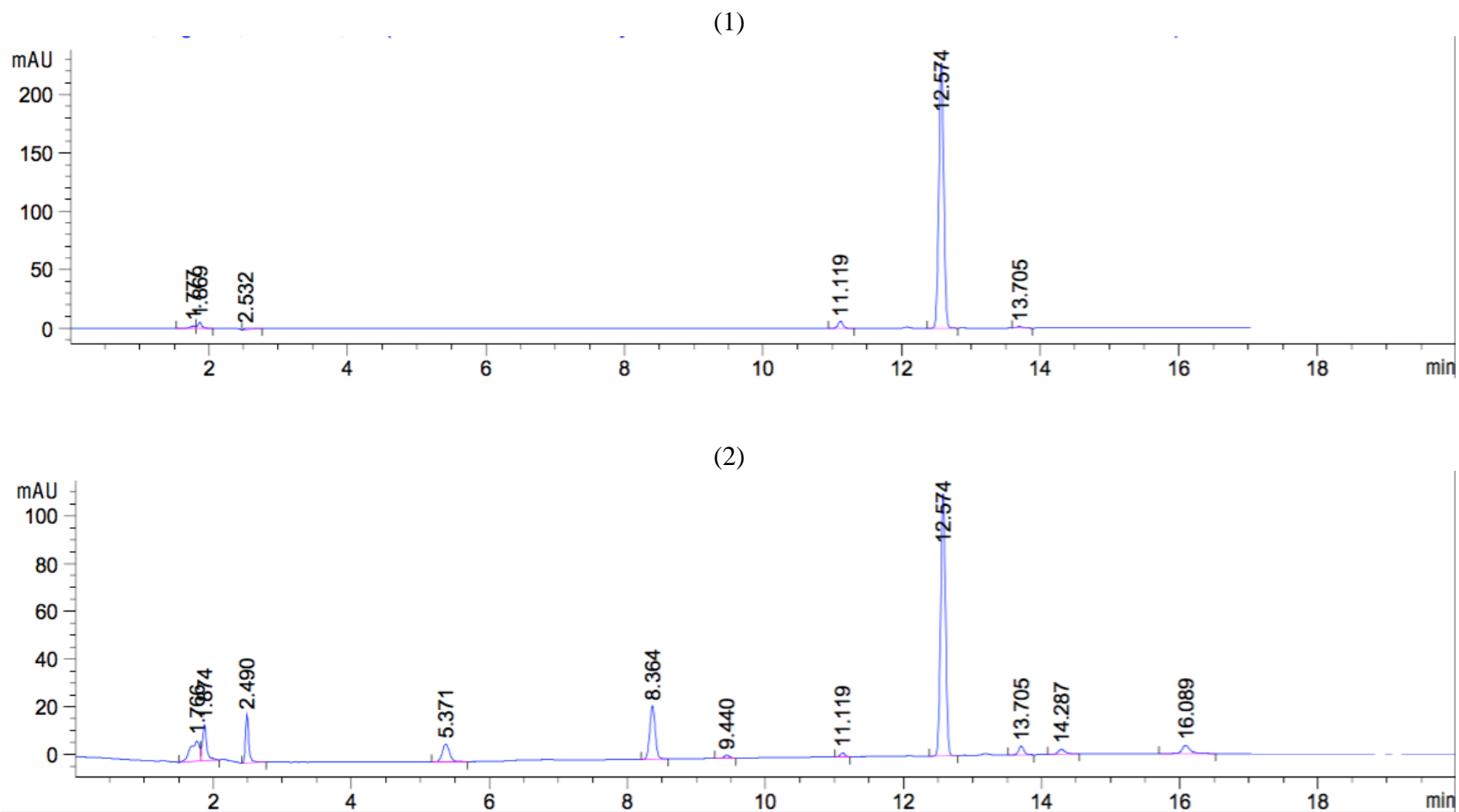
Figure A.13. HPLC spectra of DCNA at t=4 hours in artificial seawater. (1) DCNA degradation at 4 hours of irradiation measured at 380nm. (2) The formation of the products at 5.371 minutes and at 8.364 minutes measured at 254nm.
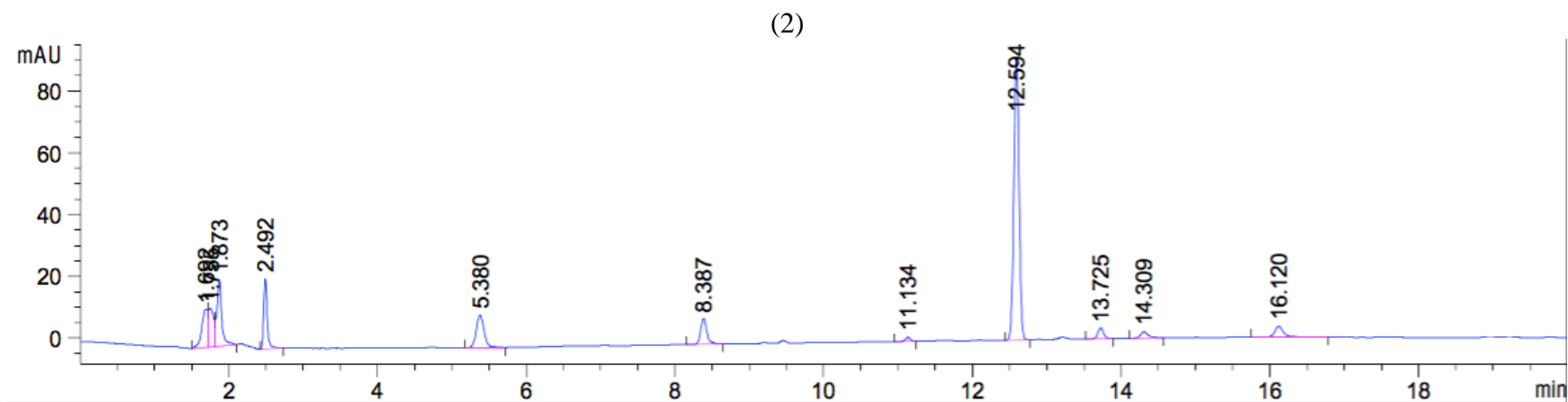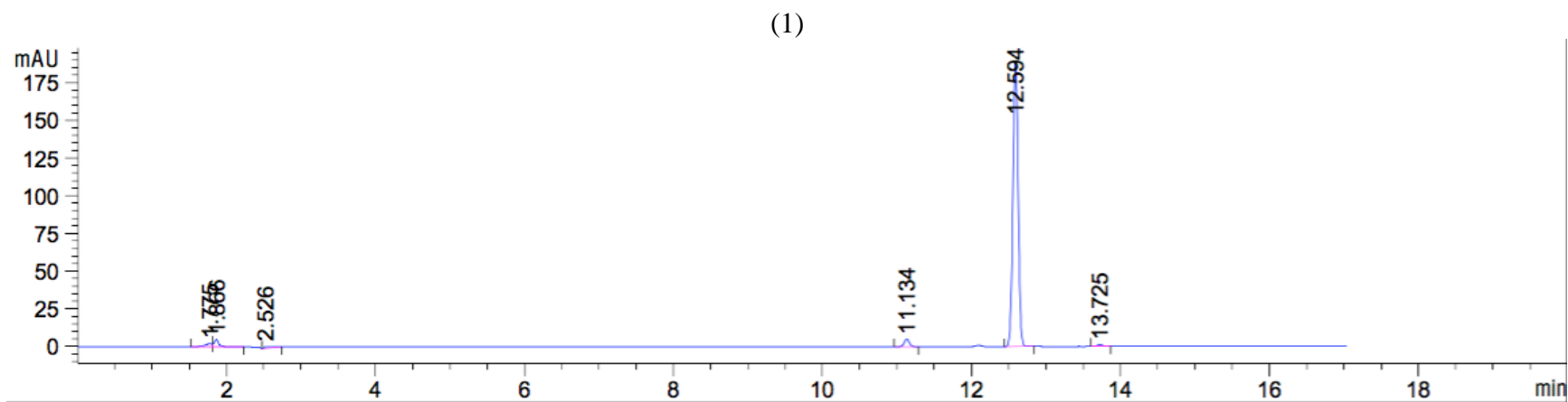
Figure A.14. HPLC spectra of DCNA at t=6 hours in artificial seawater. (1) DCNA degradation at 6 hours of irradiation measured at 380nm. (2) The formation of the product at 5.380 minutes and the degradation of the product at 8.387 minutes measured at 254nm.
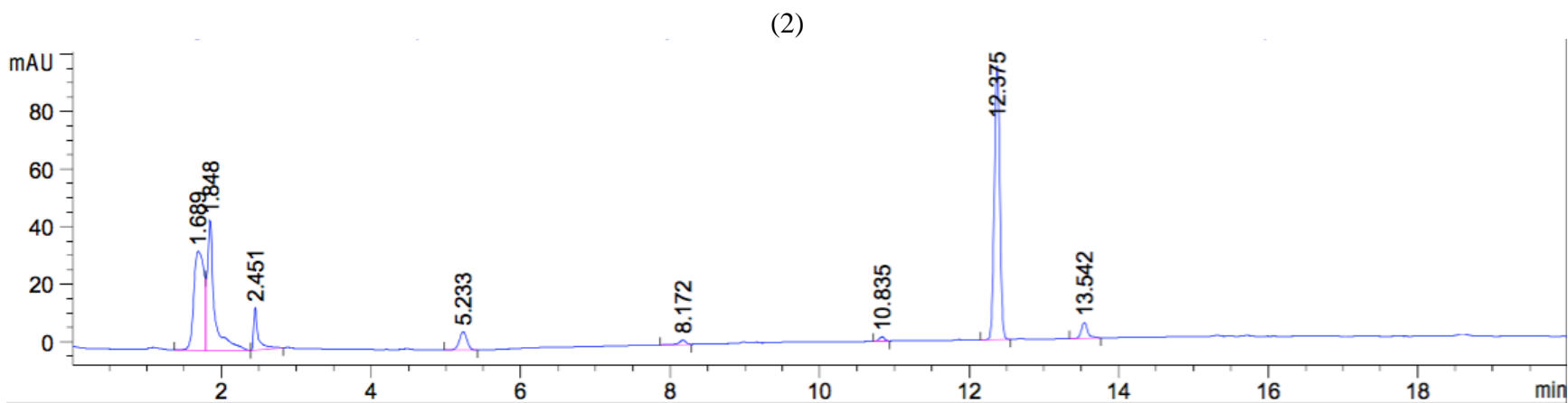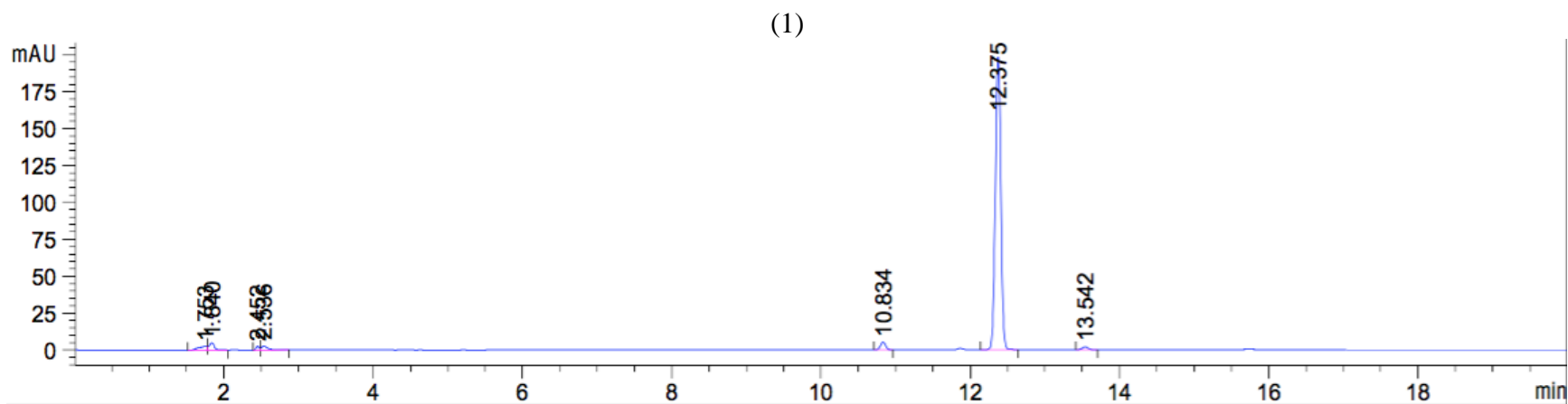
Figure A.15. HPLC spectra of DCNA at t=12 hours in artificial seawater. (1) DCNA degradation at 12 hours of irradiation measured at 380nm, at 12.375 minutes. (2) The degradation of the products at 5.233 minutes and at 8.172 minutes measured at 254nm.
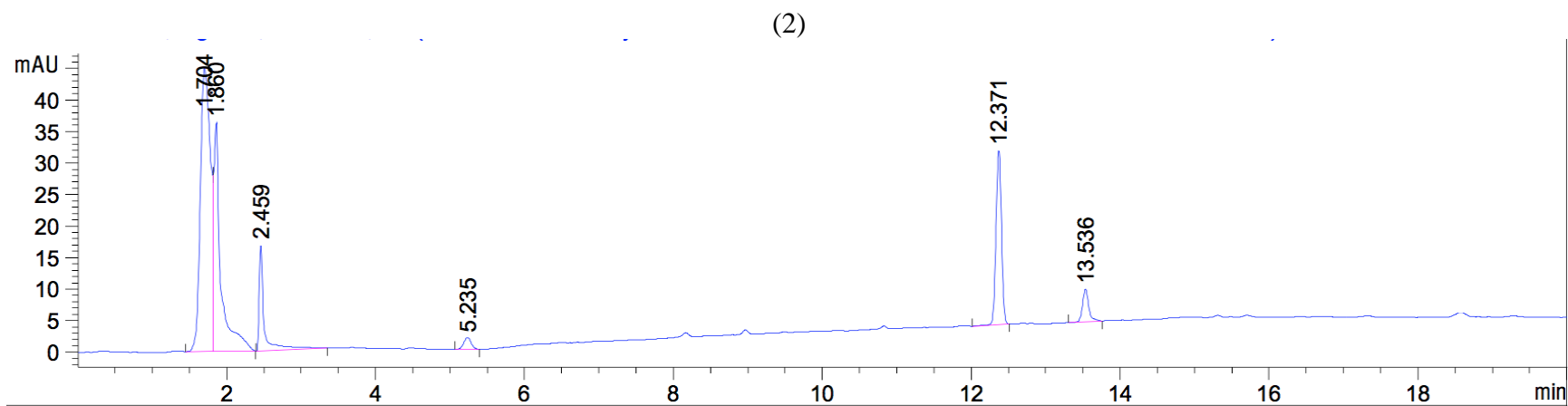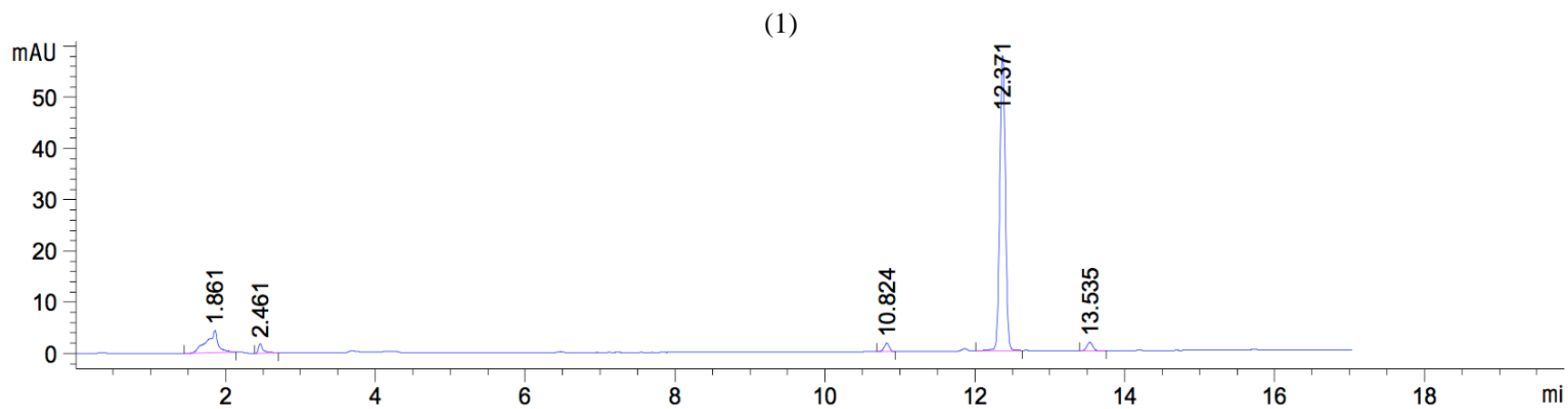
Figure A.16. HPLC spectra of DCNA at t=24 hours in artificial seawater. (1) DCNA degradation at 24 hours of irradiation measured at 380nm. (2) Shows the degradation of both products is no longer measureable after 24 hours of constant irradiation measured at 254 nm.

Figure A.17. UV spectrum for 2,6-dibromo-4-nitroaniline (DBNA).
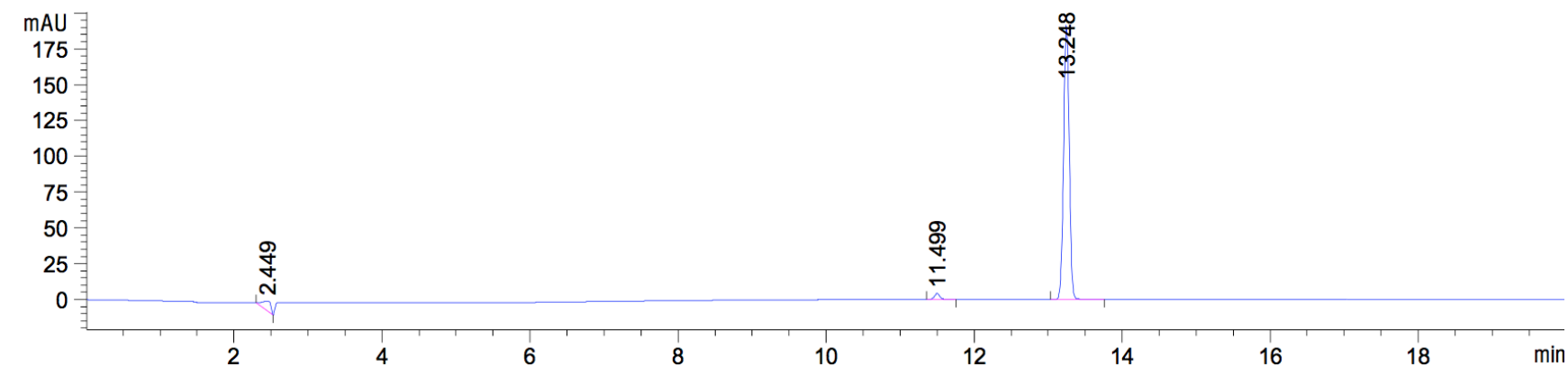
Figure A.18. HPLC spectrum of DBNA at t=0 hours in distilled water.13.248 minutes measured at 380 nm.
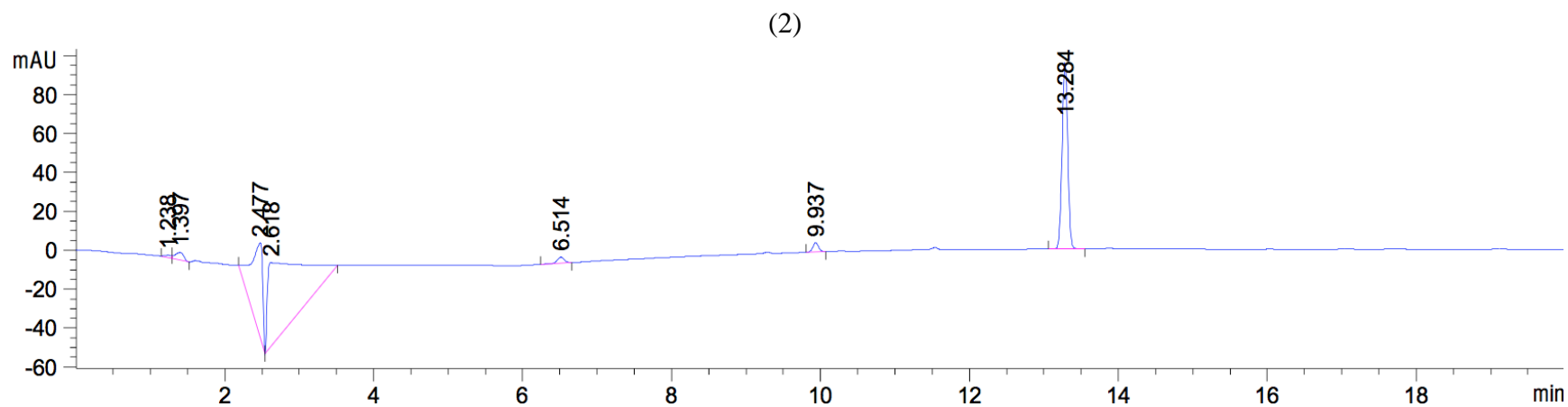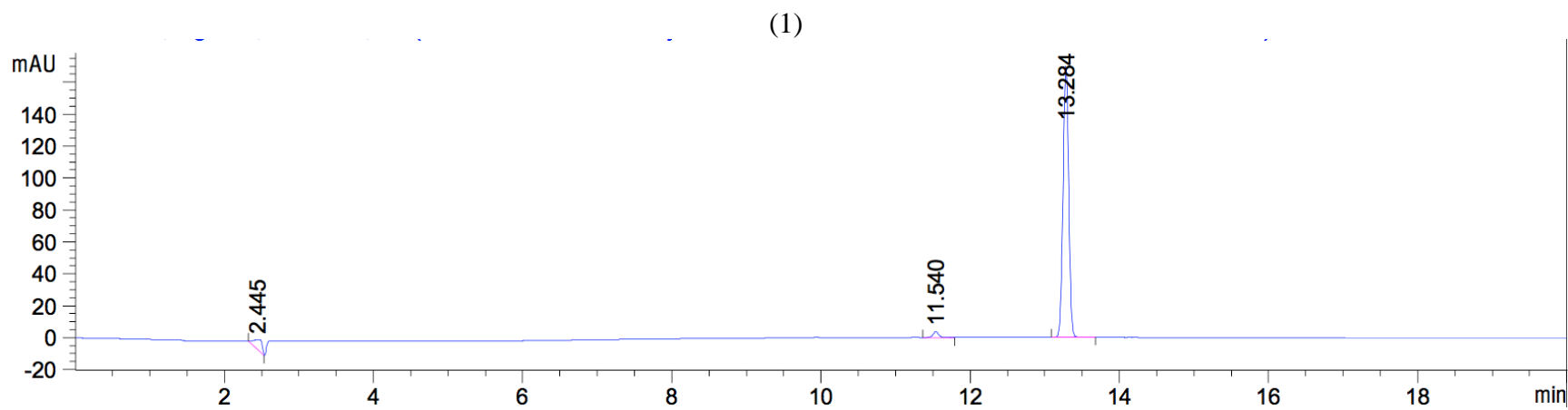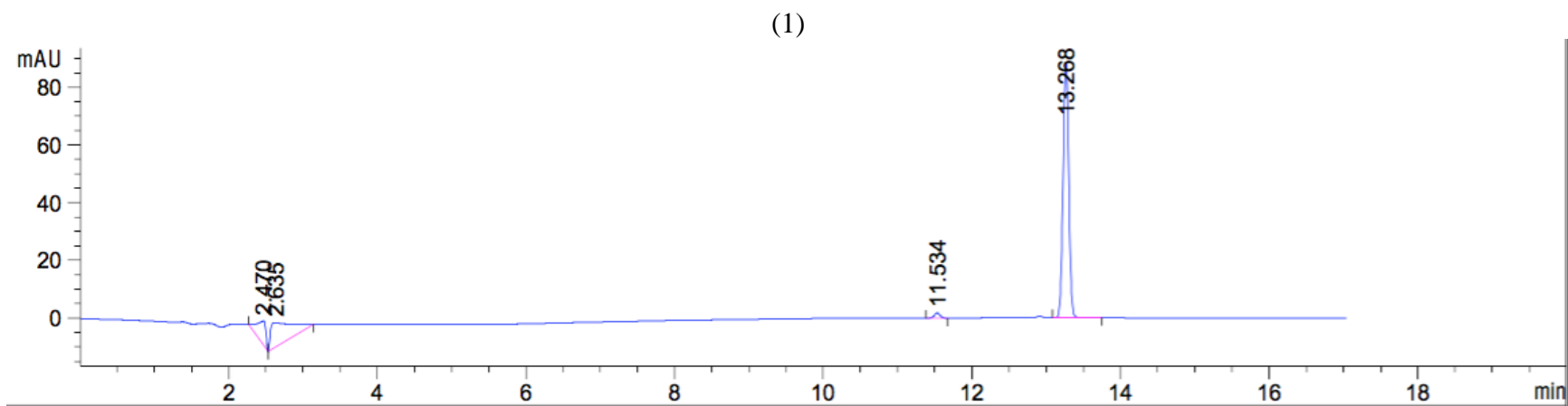
Figure A.19. HPLC spectra of DBNA at t=2 hours. (1) The degradation of DBNA after 2 hours of irradiation at 13.284 minutes at 380nm. (2) Shows the formation of photoproducts of the photodegradation of DBNA at 6.514 minutes and 9.937 minutes measured at 254nm.
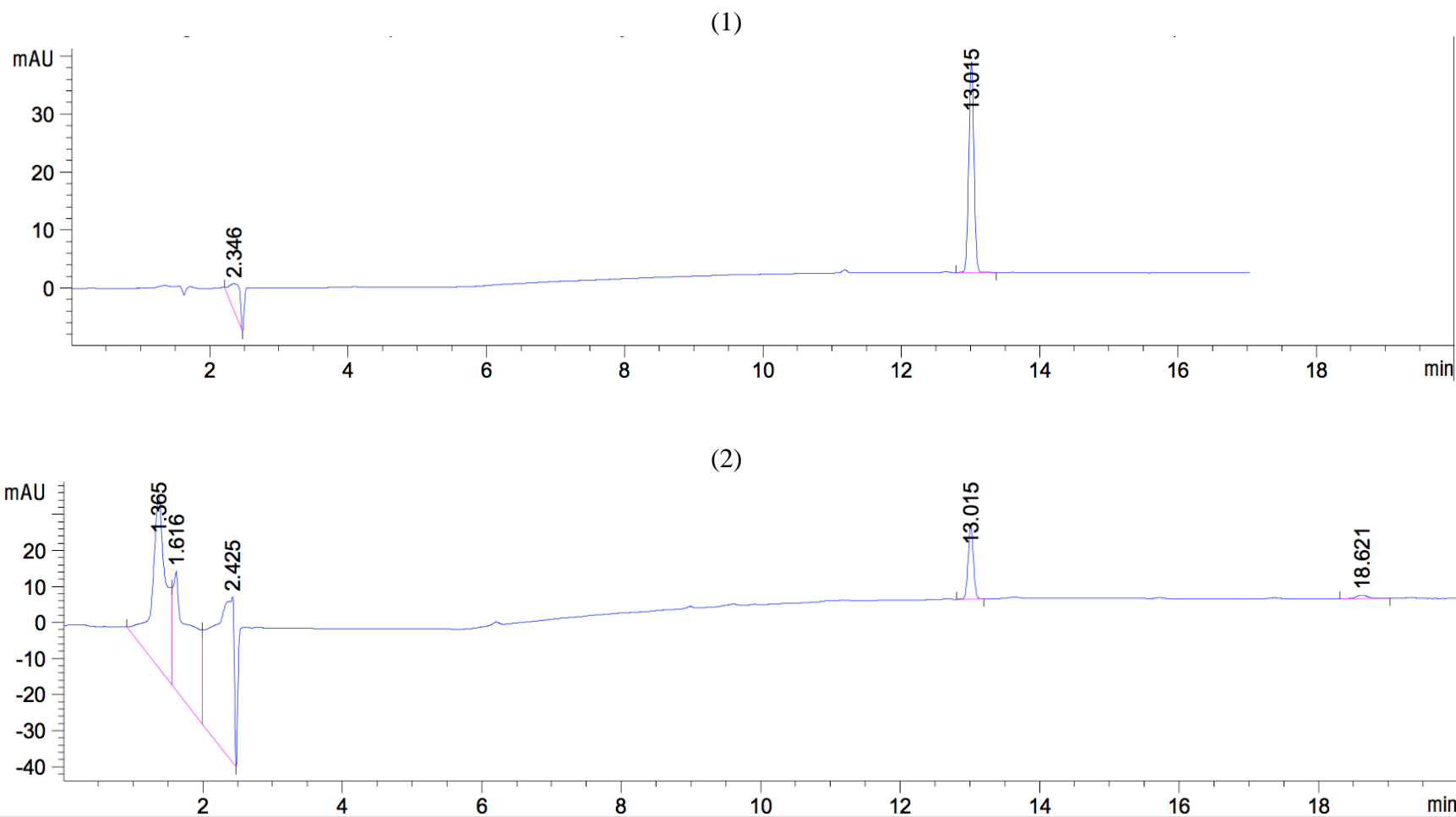
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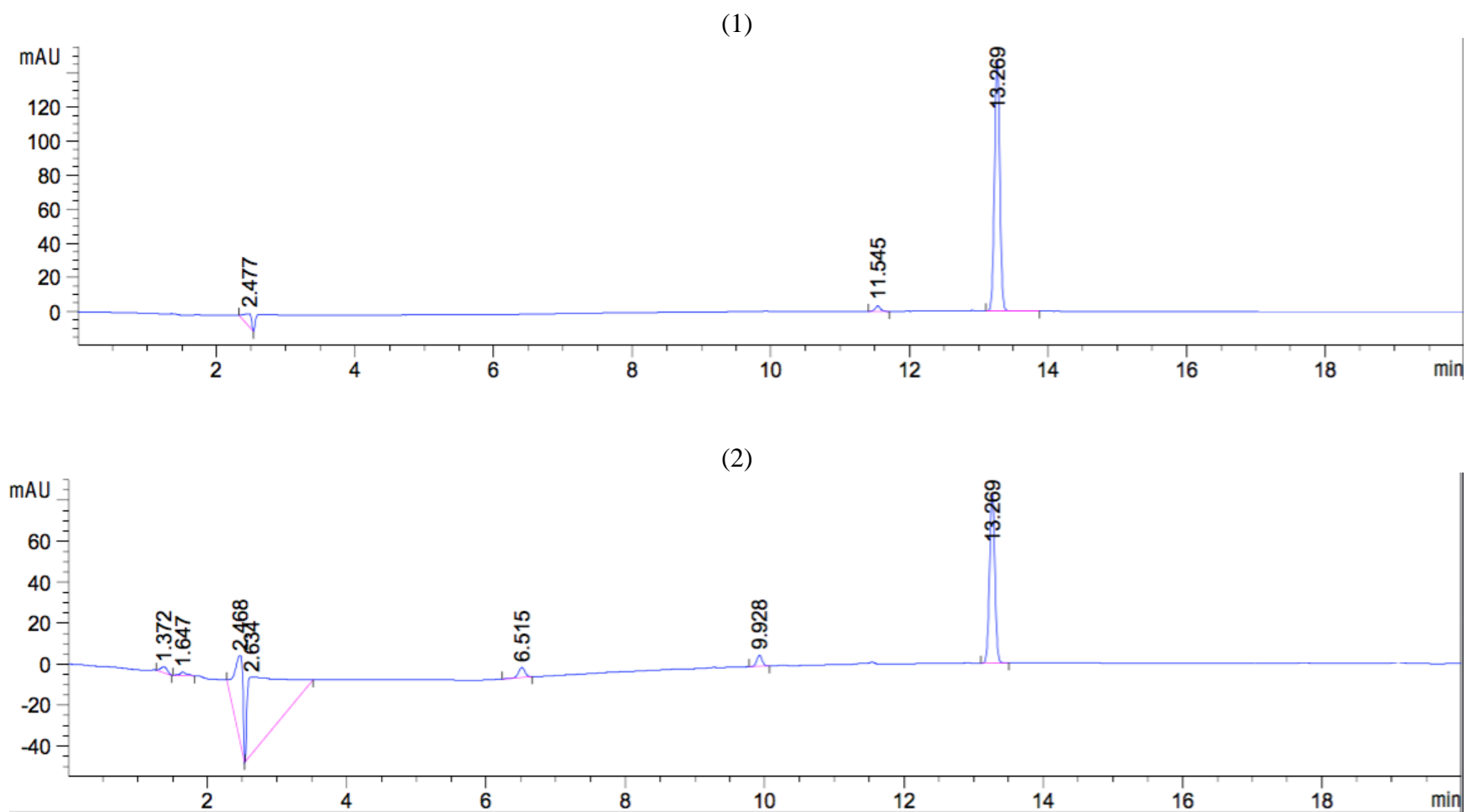
Figure A.20. HPLC spectra of DBNA at t=4 hours. (1) The degradation of DBNA after 4 hours of irradiation at 13.269 minutes at 380nm. (2) Shows the formation of photoproducts of the photodegradation of DBNA at 6.515 minutes and 9.928 minutes measured at 254nm.
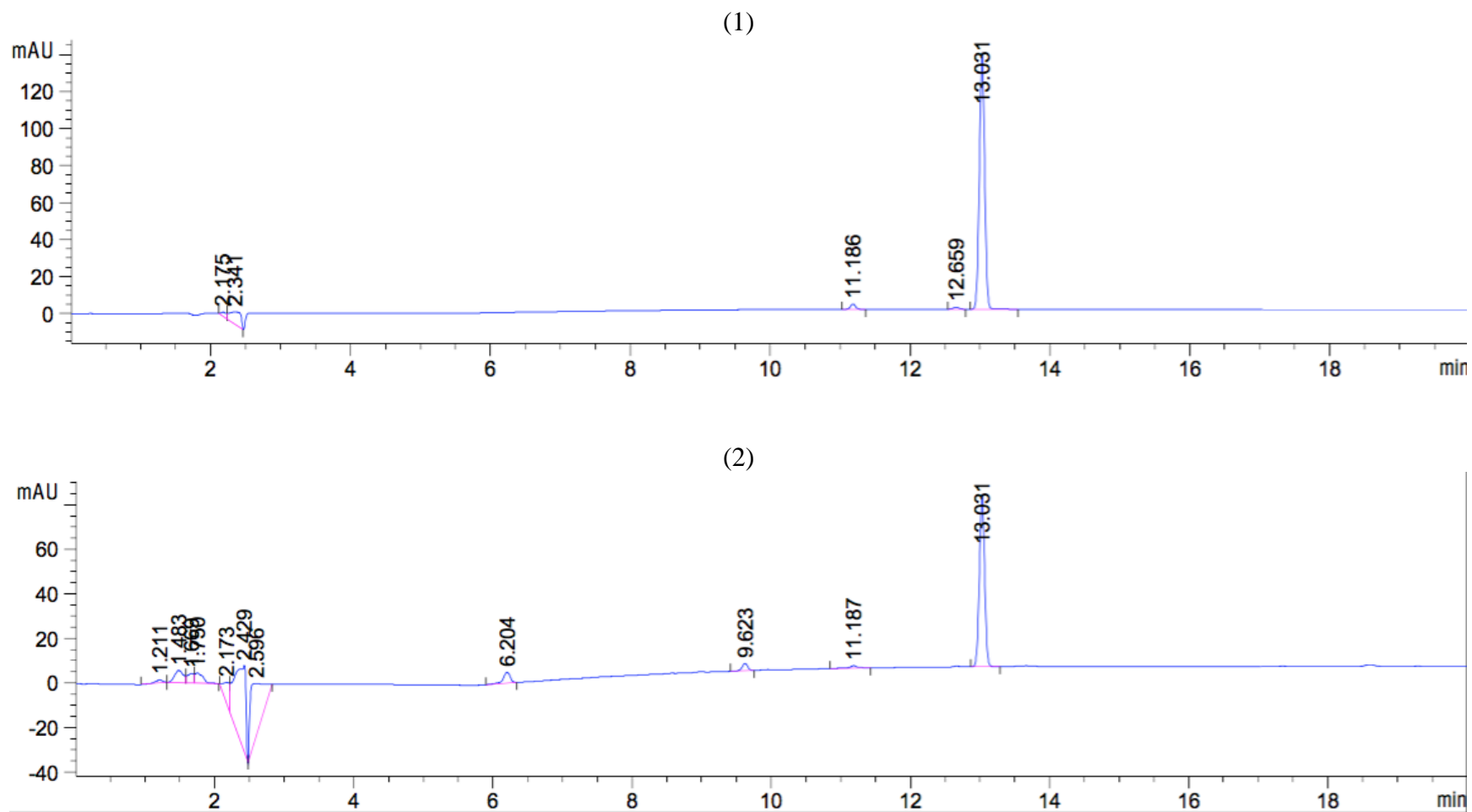
Figure A.21. HPLC spectra of DBNA at t=6 hours. (1) The degradation of DBNA after 6 hours of irradiation at 13.031 minutes at 380nm. (2) Shows the formation of photoproducts of the photodegradation of DBNA at 6.204 minutes and 9.623 minutes measured at 254nm.
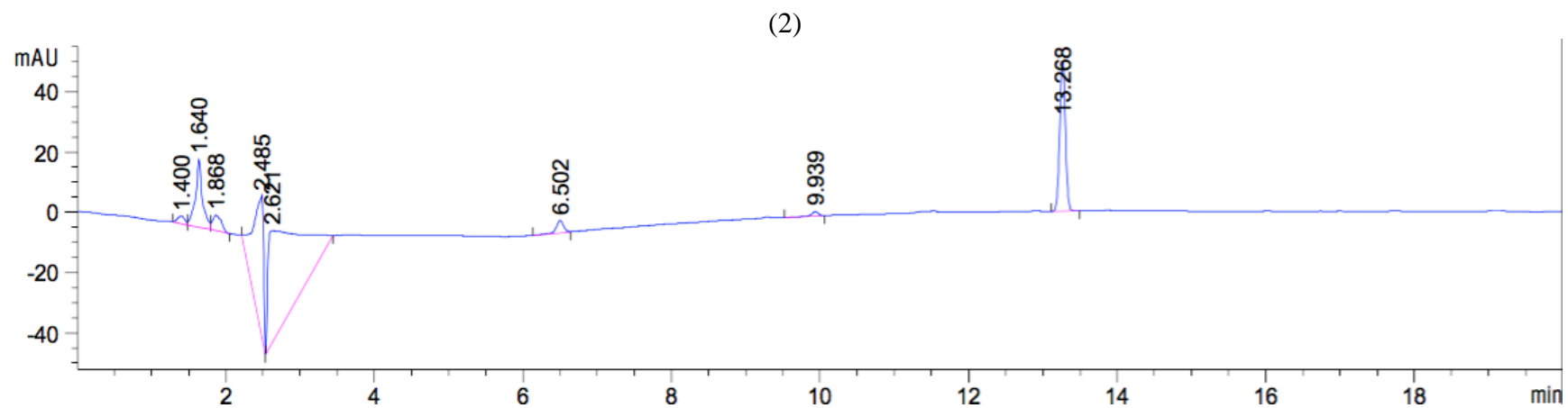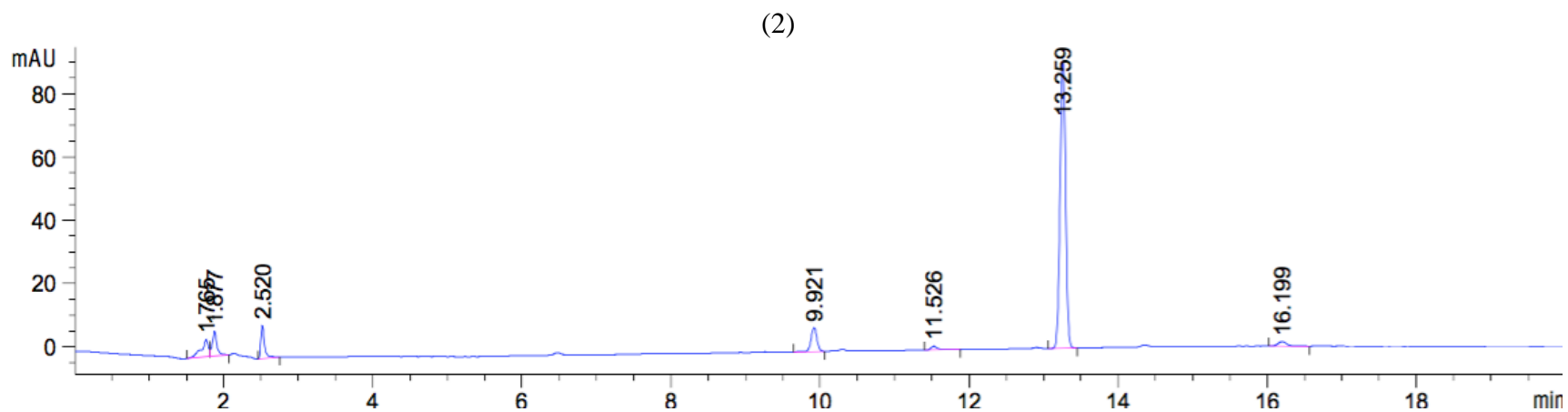
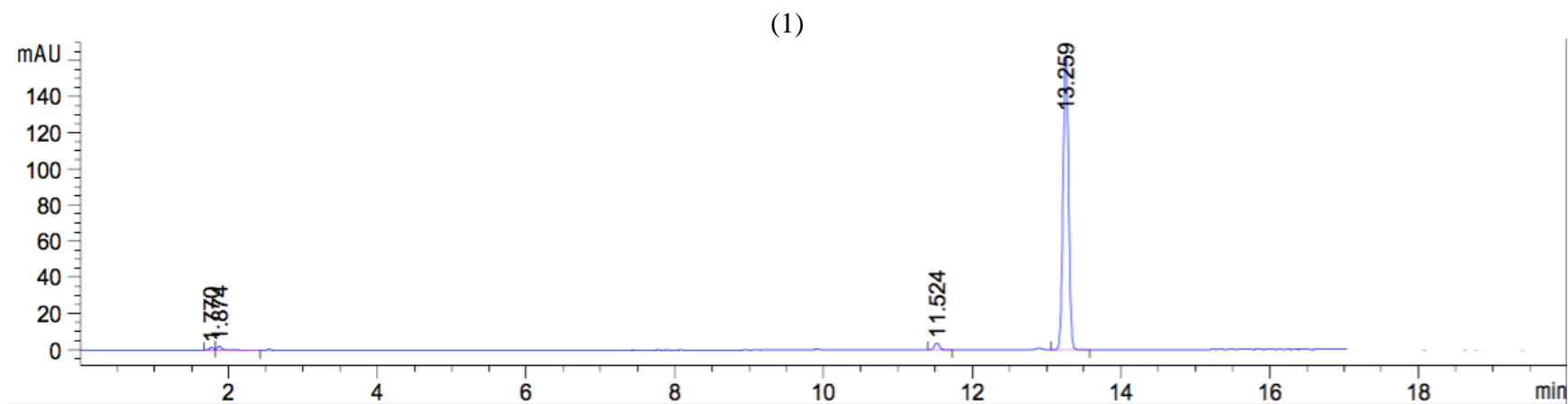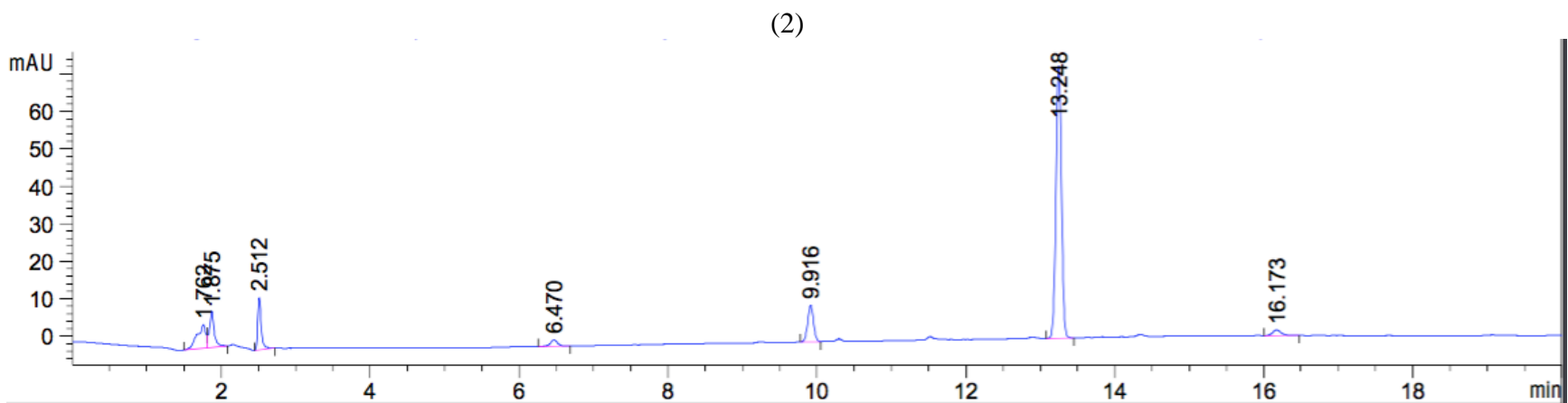Figure A.22. HPLC spectra of DBNA at t=12 hours. (1) The degradation of DBNA after 12 hours of irradiation at 13.268 minutes at 380nm. (2) Shows the photoproducts of the photodegradation of DBNA at 6.502 minutes and 9.939 minutes measured at 254nm.

Figure A.23. HPLC spectra of DBNA at t=24 hours. (1) The degradation of DBNA after 24 hours of constant irradiation measured at 380nm. (2) Shows that no photoproduct of the degradation of DBNA exists after 24 hours measured at 254nm.
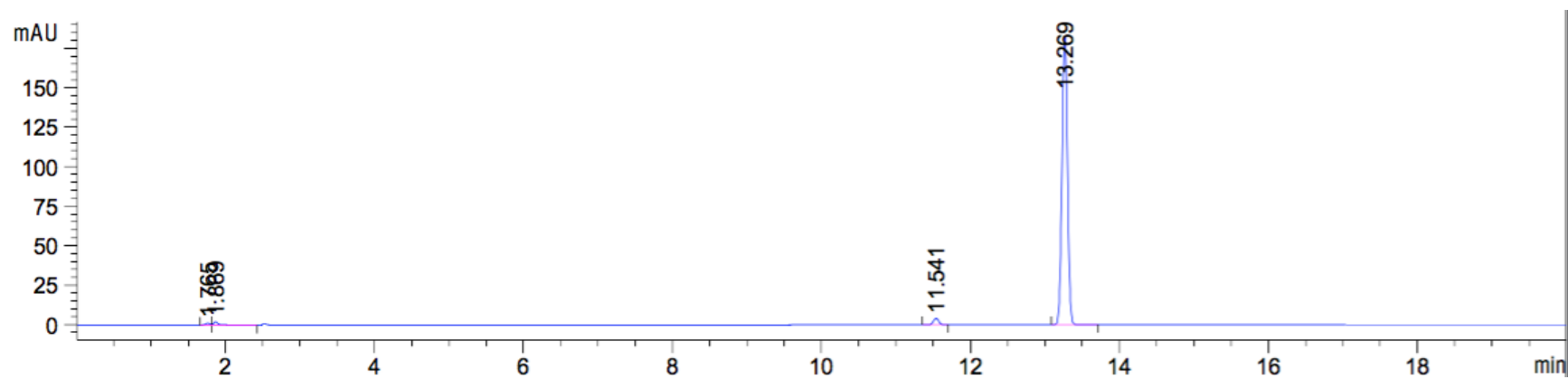
Figure A.24. HPLC spectrum of DBNA at t=0 hours in artificial seawater. 13.269 minutes at 380nm.

Figure A.25. HPLC spectra of DBNA at t=2 hours in artificial seawater. (1) The degradation of DBNA after 2 hours of irradiation at 13.259 minutes at 380nm. (2) Shows the formation of one photoproduct of the photodegradation of DBNA at 9.921 minutes measured at 254nm.
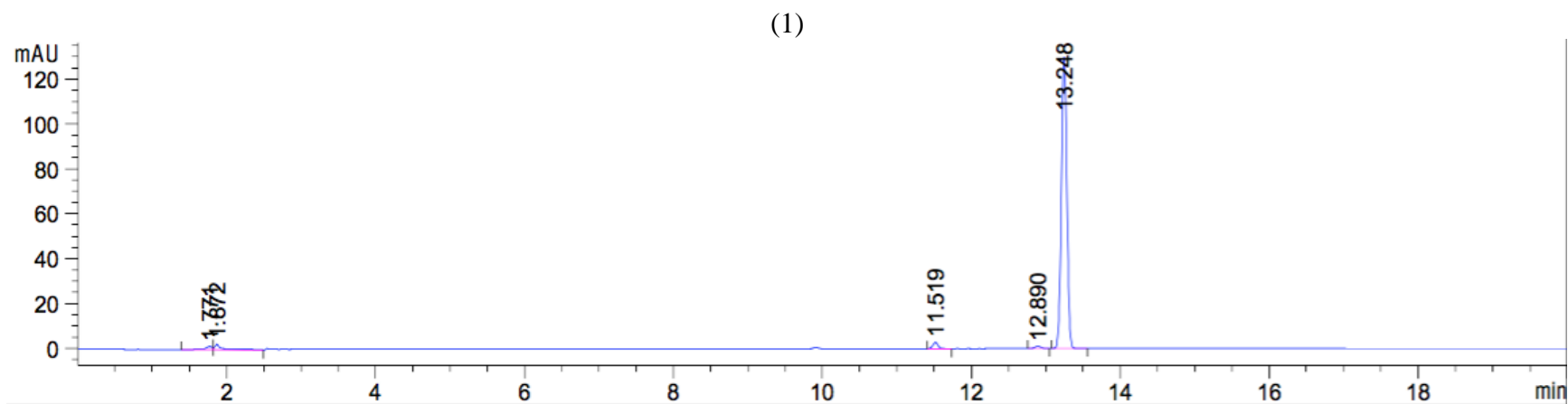
Figure A.26. HPLC spectra of DBNA at t=4 hours in artificial seawater. (1) The degradation of DBNA after 4 hours of irradiation at 13.248 minutes at 380nm. (2) Shows the formation of photoproducts of the photodegradation of DBNA at 6.470 minutes and 9.916 minutes measured at 254nm.
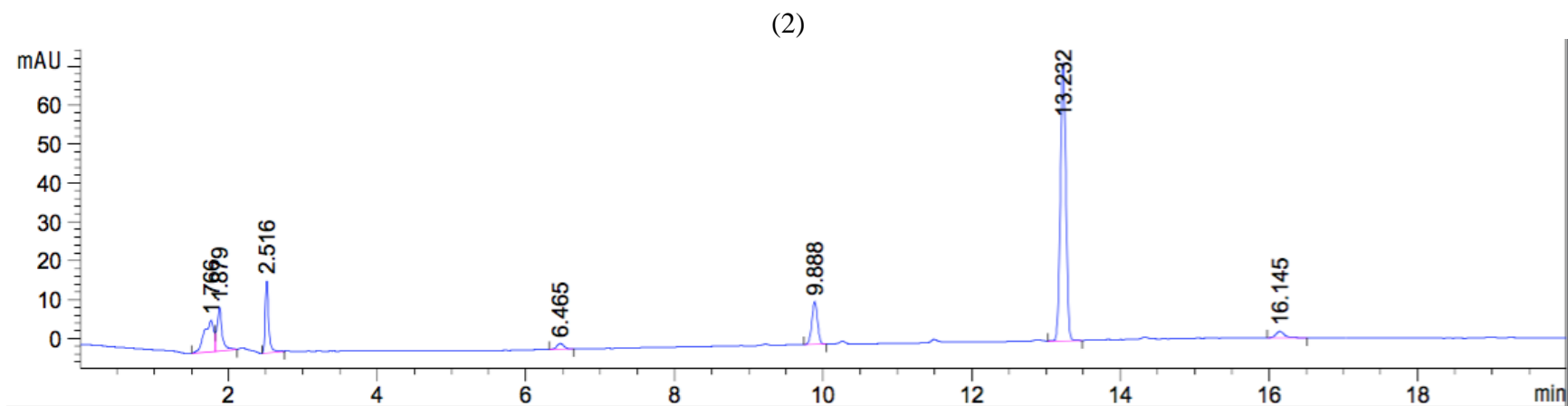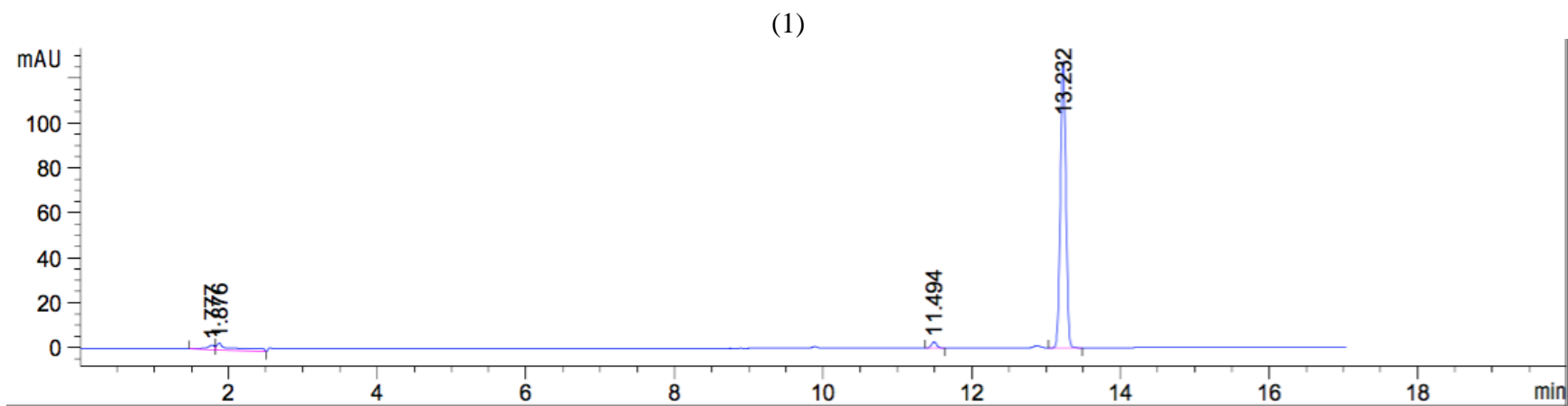
Figure A.27. HPLC spectra of DBNA at t=6 hours in artificial seawater. (1) The degradation of DBNA after 6 hours of irradiation at 13.232 minutes at 380nm. (2) Shows the formation of photoproducts of the photodegradation of DBNA at 6.465 minutes and 9.888 minutes measured at 254nm.
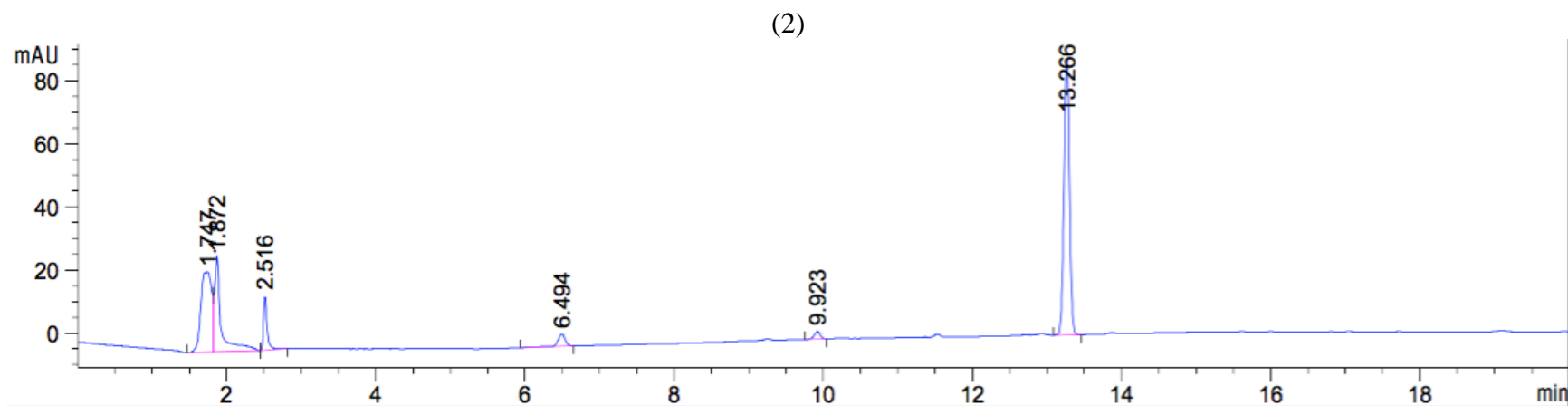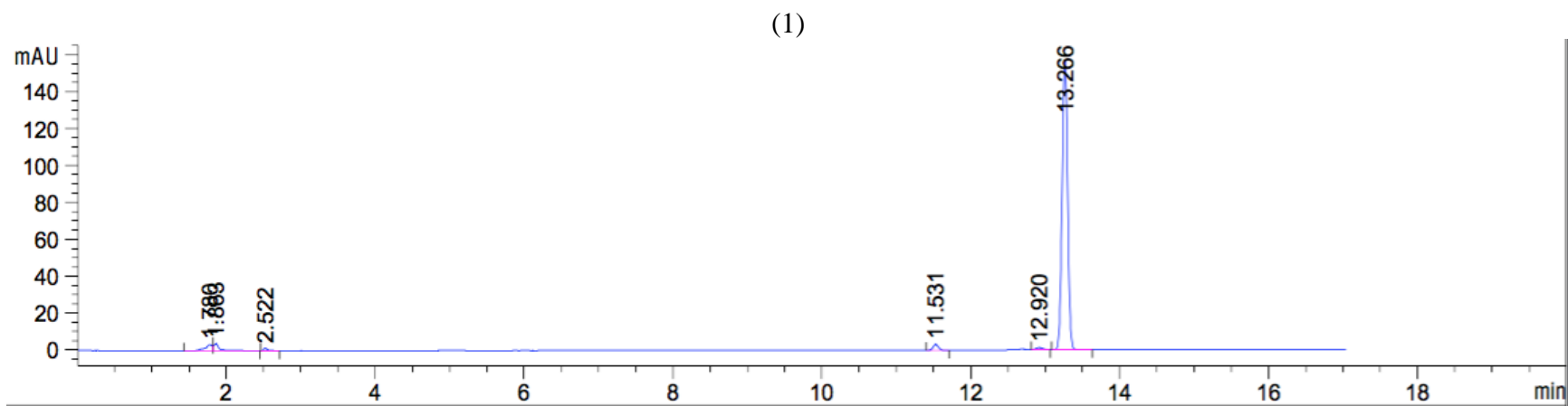
Figure A.28. HPLC spectra of DBNA at t=12 hours in artificial seawater. (1) The degradation of DBNA after 12 hours of irradiation at 13.266 minutes at 380nm. (2) Shows the degradation of photoproducts of the photodegradation of DBNA at 6.494 minutes and 9.923 minutes measured at 254nm.
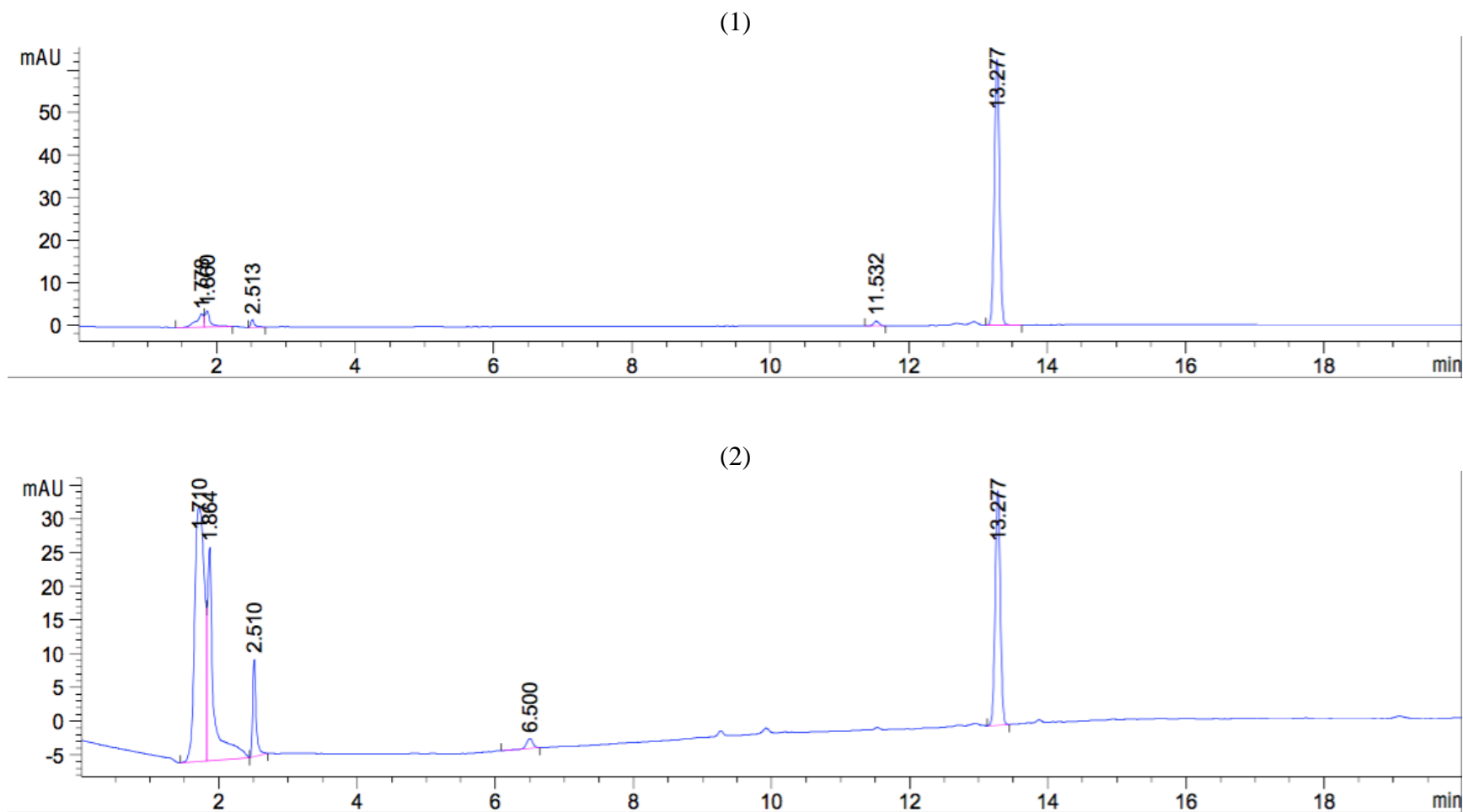
Figure A.29. HPLC spectra of DBNA at t=24 hours in artificial seawater. (1) The degradation of DBNA after 24 hours of irradiation at 13.277 minutes at 380nm. (2) Shows the degradation of photoproducts of the photodegradation of DBNA, with only a small amount of the product at 6.500 minutes remaining.

VITA

Emily Noelle Vebrosky, of Frackville, Pennsylvania, received her bachelor's degree from Lycoming College in Williamsport, Pennsylvania in Chemistry in 2014. She began her master's studies at Louisiana State University in the Department of Environmental Sciences in the fall of 2014. Entering undergraduate intending to study environmental chemistry, her interest in environmental science continued to grow after interning at Lycoming College's Clean Water Institute focusing on stream and river impairment within the Susquehanna River Basin and monitoring trout populations throughout Pennsylvania; these interests eventually lead her to Louisiana. Emily plans on continuing her research and education at LSU.