The Analysis of Ballpoint Inks with APCI-MS after Fading with Light, Hydrogen Peroxide and Sodium Hypochlorite Bleach

by

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A thesis submitted in partial fulfilment for the requirements for the degree of Doctor of Philosophy at the University of Central Lancashire

September 2015

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Acknowledgments

I would like to thank my supervisors Dr Allison Jones and Dr Richard McCabe for their continued support and guidance over the last 7 years. I would also like to thank the School of Forensic and Investigative Sciences for funding this project. I must also thank Professor Jaipaul Singh who offered much guidance, advice and support, especially at annual progression and Clare Altham in the Graduate Research Office who assisted in the many administrative processes.

This project would not have been possible without the technical support of Sal Tracy, Jim Donnelly and Tamar Garcia. Sal you could fulfil my requests for chemicals even at short notice and thank you for the use of the lab when I had nowhere else to go. Jim and Tamar, thank you for your assistance with the LCMS, it's been tough! It was much better in the old days in Maudland building when it was just me using it. Thank you also for your patience and finding me a cupboard in the analytical unit for all of my samples. You'll get your cupboard back soon.

I have to thank my colleagues; Karen Lupton, Adam Wilcox, Izzy Colclough and Alan Scott who in the final months took the burden off me by taking some of my marking, it very much appreciated.

Dan Clark and Cat Tennick, it is both your faults, persuading me to do a PhD, but what fun times we had, four of us sharing a box of an office. Well it is just me now and nearly the end of an era. Thank you especially Cat for being my proof reader.

To my family, this has been a long journey for you too. I really appreciate all the love and support that you have given me. Thank you for believing in me and giving me the strength to carry on when things weren't going quite to plan. To all my friends, thank you for the supplies of chocolate and energy drinks, they were definitely a saving grace, but it's a good job that I'm finishing because I really need to get back to the gym.
Thank you all for your patience. I have not seen a lot of you for many months now but I will make it up to you I promise.

Thank you especially to John Cotterill, for being my chauffeur at stupid times of the day and night, for your encouragement, support and understanding. You have been there through the highs and lows and have made it out the other side. Thank you for your time consuming “monkey work” on my excel files, it saved me weeks, just a shame there wasn’t more you could do!

For the many people I have not mentioned by name, THANK YOU

Abstract

The ability to discriminate between different inks and to determine the length of time an ink has been on a substrate can provide important scientific evidence, especially in cases involving document fraud. Many techniques have been used to analyse inks for ink dating including chromatography and spectroscopy, but the results are unreliable as a result of factors affecting the aging process such as light. This study utilises established techniques in Forensic Document Examination, including filtered light examination but also novel techniques for ink analysis; Atmospheric Pressure Chemical Ionisation (APCI) to analyse inks and dyes with the aim of discriminating between samples based on their degradation products. APCI-MS was used for the first time to study nineteen ballpoint pens from a range of manufacturers by investigating the chemical processes that occur and the products that are formed following the deposition of ink onto a substrate and in solution. Monitoring the degradation process as an ink ages and fades enables the identification of components present in the inks. Using molecular mass data, accurate ink component identifications could be made over a period of two years on samples subjected to a range of external influences. Light, hydrogen peroxide and sodium hypochlorite bleach were used to simulate natural and deliberate fading of inks and dye solutions. Benzophenone and phenol molecules were identified as degradation products but their presence differed for each of the different conditions tested such as no phenol products when bleach was used. This novel approach to ink analysis utilises existing equipment commonly used by document examiner to analyse inks that are old or faded in some way, in order to discriminate between the inks or determine method of alteration.

Chapter 1 Introduction

1.1 Forensic Science

Scientific evidence can potentially be obtained from any item that is found at a crime scene, on a victim, on a suspect, in a car, or at any location that could be linked to a crime. Scientific evidence is extremely important in criminal investigations, as it can provide the investigating team with information about the chronology of events, possible motives, any weapons used and potentially who the offender is. Scientific evidence can be crucial in the decision making that takes place to reach a guilty or non-guilty verdict. However, it is of the utmost importance that all scientific evidence is obtained in accordance with strict protocols and that any subsequent analyses are appropriate and accurate, so that the integrity of the evidence cannot be put into question. Careful consideration of types of analyses that are carried out is important due to the potential that one analytical protocol may have to interfere with any subsequent tests. If possible, the best procedure to carry out is one that is non-destructive to the sample. This then allows re-examination or further analyses of the evidence, such as DNA profiling or fingerprint examination.

1.1.1 Document Analysis

The application of allied sciences and analytical techniques to solve questions concerning documents is termed forensic document examination. Documents under examination are called questioned documents and can involve the analysis and comparison of questioned handwriting, typewriting, printing, papers, inks, photocopied documents and other documentary evidence. These can be in many different forms, including letters, envelopes, currency, cheques, passports, contracts and wills. In many cases the questioned document is compared with materials of a known origin in an attempt to establish its authenticity or to detect any alterations. Science is increasingly used in court and is an important part of the criminal justice system. In one year alone, over 150,000 cases were dealt

with by forensic science providers covering all areas of forensic science.[1] This has created a growing need for more diverse ways to obtain and analyse such materials.

Handwritten entries are present on a large number of documents. Many printed and legal documents, such as wills and contracts, require a signature and therefore will contain writing ink. Other formal documents, such as cheques and receipts usually contain an area filled in by hand using a writing implement. Informal documents are frequently entirely handwritten, e.g. diaries, letters and notes. There are therefore, many documents containing writing ink, which could be altered or forged.

The analysis of ink can be very important in cases involving the examination of questioned documents, e.g. subtle alterations to documents such as tax returns, wills and insurance claims can have significant financial implications.[2] Ink analysis generally comprises three types of examination; comparing two or more inks to determine if they could have come from a common source, identifying ink on a document to determine the potential source of the ink and establishing when the written entries were made to authenticate the date of the document. The ability to differentiate between inks is of great importance in forensic science because it allows an evaluation of the authenticity of a suspicious document.[3] Common forgery includes modifications to information, such as dates or values and insertions to the original writings. This type of forgery can be identified by showing that the questioned ink entry was created with a pen containing a different ink from that used to make the original entries or by dating both the original and newer ink writings.[4] Other methods include chemical erasure using common products such as hydrogen peroxide or household bleach (sodium hypochlorite).

The detection of alterations or additions to a document and the determination of factors such as the age of an ink can be both contentious and challenging problems for document examiners.[2] Experiments have shown that factors such as time, exposure to light and temperature have been shown to influence the apparent age of a document.[5] All of these factors and the conditions under which the document have been stored, are usually unknown to the examiner and this

creates a major challenge to an examiner trying to provide the exact date when the document was prepared.[6-8]

Lots of research has been dedicated to the dating of documents and inks, such as microspectrophotometry,[9] IR spectroscopy,[10] GCMS,[11] but nothing to date has been published that focuses on old and faded documents from natural and artificial fading and trying to discriminate between the samples by specifically looking at the degradation products. That is the aim of this thesis.

1.1.1.1 Paper

The most common substrate used with writing and printing inks is paper. Paper is composed of cellulose fibres obtained mainly from wood, with the addition of a variety of inorganic fillers which are used to improve various properties (e.g. whiteness, ink absorption, reflectivity and stiffness) together with sundry organic materials used as sizes and colouring agents.[12] Figure 1.1 shows the basic structure of cellulose.

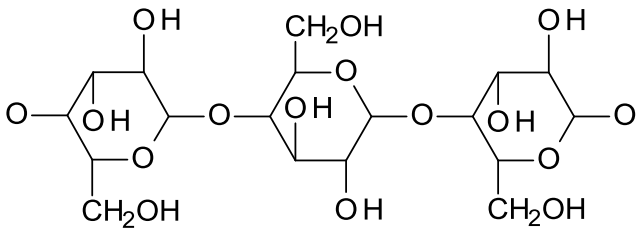


Figure 1.1 Cellulose Structure

Table 1.1 shows common fillers used in the paper industry and their properties relevant to paper.

Table 1.1 Common Fillers used in Paper Manufacturing

Filler	Formula	Properties
China Clay (Kaolin)	$Al_2O_3.2SiO_2.2H_2O$	Produces a smooth surface
Calcium Sulphate (Gypsum)	$CaSO_4$	Produces fine fibres leaving a lower density and therefore lighter paper
Calcium Carbonate	$CaCO_3$	Low acidity so good for papers requiring high durability
Magnesium Silicate	$MgO.SiO_2$	Produces a smooth surface
Barium Sulphate	$BaSO_4$	Opacifier –increases the opacity of the paper
Titanium Dioxide	TiO_2	Opacifier
Zinc Sulphide	ZnS	Opacifier

Different paper manufacturers use different additives to modify the properties of their products and consequently the composition of paper varies considerably. This is an important factor when analysing the reactions of ink on paper.
The factors mentioned previously that can affect a document, can have a specific effect on the paper itself. The aging of paper is due to the degradation of the cellulose substrate and this is also an important point to be considered when carrying out examinations on paper based documents.[13]

1.2 History of Writing Ink

Writing inks are believed to have been developed around 2700 B.C. From the first century A.D. Indian inks were widespread and consisted of a mixture of carbon and glue or vegetable gum.[14] These inks were replaced by iron gallotannates in the 12th century.[14] Originally iron gallotannate inks were colourless but then turned black following oxidation on the paper substrate. In 1834 a blue dye was added to the ink enabling them to be visualised and it is these inks which formed the basis of fountain pen inks. Fountain pens were the main writing implement for many years. There is evidence which shows the

ballpoint pen was first constructed in 1895 and the ink patented in 1898, but it was not until the 1930's that the ballpoint pen was introduced to Europe where 25,000 ballpoint pens were produced between 1935 and 1939.[15] It took until 1945 for the ballpoint pen to be made available to purchase in the USA by Biro.[15] Fibre tip pens were introduced in 1962.[16] The inks used in fibre tip pens are of a different composition to that used in ballpoint pens, in that they are water-based. The roller ball pen was developed in 1968 and again contains a different ink to ballpoint pens, but a similar ink to fibre tip pens.[16] The most recent pen, made available in the 1990's is the gel pen.[16] As the name suggests, the ink is in a gelatinous state, rather than a liquid and pigments are used instead of dyes to create the colour. The gel pen has been developed due to a world-wide need for environmentally friendly writing instruments containing little or no hazardous materials such as heavy metals in the pigments or resins that are toxic.[17] Although a wide range of writing materials are now available, ballpoint pens are still the most popular pen used by the population, it is the most widely used instrument for writing on paper and previously 80% of casework requiring ink analysis contained ballpoint pen ink.[18-20] For this reason ballpoint pen ink was chosen to be the focus of this study.

1.2.1 Ballpoint Pen Ink Composition

Ballpoint pen ink is viscous and contains three main components; a component to provide colour, (usually organic dyes), a solvent (also known as the vehicle) and a number of additives.[21,22] The vehicle constitutes approximately 50% of the ink, dyes another 25% and additives make up the final 25%.[23] These components define the ink's individual properties which vary depending on its intended final use,[24] giving rise to a variation in the additives used and their relative proportions. Altering the components during the manufacturing process produces the desired properties which may include varying degrees of tackiness, light fastness and colour. Other ingredients may also be present in small quantities, such as lubricants, biocides, surfactants, such as alkylamines and pH adjusters, for example aryl guanidines. Also viscosity adjusters or corrosion inhibitors, such as organophosphates, can be added as well as antioxidants such as butylated

phenols, softeners for ink flow ability such as phthalates and fatty acids for ball lubrication may be present.[25]

The basic composition of ballpoint ink has not changed considerably over time, but during the 1950's a major change was made to the vehicle, moving from the use of oil-based to glycol-based inks. This was driven by the fact that there was a limited range of oil soluble dyes and these dyes also tended to fade quickly.[14] Knowledge of such changes in composition can be useful when trying to date an ink, but this historical information is only useful to a point.[26] The increased demand for writing instruments has generated a wide variety of ink formulations, which may contain tens of chemical components.[27]

1.2.1.1 Dyes

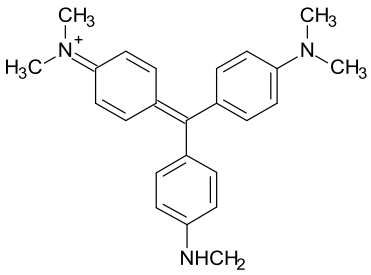
Dyes are used to provide colour to a range of products including medication, food, clothing and are key components in paints and inks.[28,29] Dyes are normally coloured, ionic aromatic organic compounds that are soluble in organic solvents. A dye is coloured as a result of the absorption of electromagnetic radiation. This is possible because the molecule contains two components; a chromophore, which is usually a conjugated arrangement of π-orbitals and forms the main body or skeleton of the molecule and one or more auxochromes, which are electron-donating or withdrawing substituent groups used to fine tune the absorption wavelength. The chromophore is characteristic of most classes of dyes and is mainly responsible for the colour. The chromophore is a region in a molecule where the energy difference between two π and π^* molecular orbitals falls within the range of the visible spectrum. Chromophores normally consist of extended conjugated systems and usually include C=C, N=N and aromatic rings. Functional groups such as alkylamines and carboxylic acids are commonly used to extend the chromophore and are known as auxochromes as they can alter the colour of the dye by making small changes to the energy difference of the π and π^* orbitals of the chromophore.

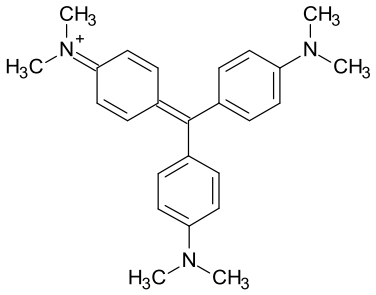
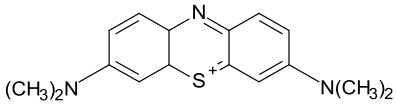
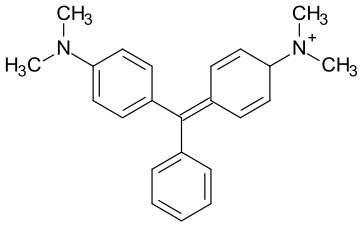
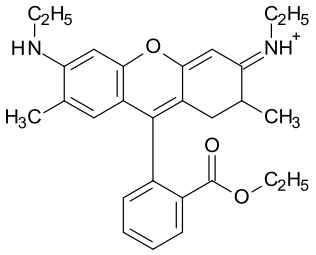
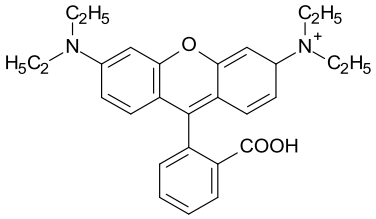
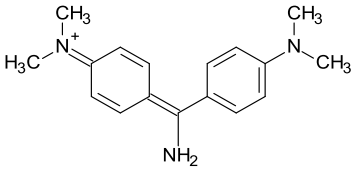
There are many different types of dyes and many different names even for the same compound. The Colour Index (C.I) was introduced in 1924 to classify dyes

according to their structure, source, colour and method of application.[30] The main classes include azo, arylmethane and natural dyes. Each dye is given a number based on its chemical structure, e.g. azo dyes have values ranging from 11,000 to 40,000 and arylmethane dyes range from 41,000 to 45,000. Such a database of numbers is useful as it can resolve issues of identity when an uncommon name is used for a dye. For example, Crystal Violet is the common name used for Basic Violet 3 (the C.I. generic name) both of which have a C.I. number of 42555.

Dyes used in ballpoint pens originate from several different groups including basic (cationic) dyes such as the tri(aminoaryl)methanes and acid (anionic) dyes derived from diazo compounds or phthalocyanines.[31,32] Triarylmethane dyes have a characteristic structure containing three aryl groups attached to a central carbon atom. Azo dyes are characterised by the presence of one or more azo bonds (-N=N-) and have many applications, they are also produced in the largest quantities.[33,34] Common dyes used in ballpoint pen inks include Basic Violet 3, Basic Blue 26, Basic Red 1 and Basic Green 4.[35,36] Chemical structures of typical triarylmethane dyes found in ballpoint inks can be seen in Table 1.2.

Table 1.2 Examples of Common Dyes used in Ballpoint Ink with their Structure and Mass

Dye	Other Name(s)	C.I Number	Structure	m/z
Basic Violet 1	Methyl Violet (2B)	42535		358

Dye	Other Name(s)		Structure	m/z
Basic Violet 3	Crystal Violet, Methyl Violet 10B	42555		372
Basic Blue 9	Methylene Blue	52015		284
Basic Green 4	Malachite Green	42000		329
Basic Red 1	Rhodamine 6G	45160		443
Basic Violet 10	Rhodamine 6B	45170		443
Basic Yellow 2	Auramine	41000		268

The colour required for writing inks is often achieved by mixing different dyes,[27] for example black inks frequently contain a mixture of blue, purple and yellow dyes.[14] The exact details of the ink formulation present in a pen or brand is important information for scientists, however this information is rarely disclosed by the manufacturing companies for proprietary reasons.[37] It is believed that each brand uses their own unique formulation for their inks.

Triarylmethane dyes are popular dyes used in inks due to their low cost and strong colours, however they are characterised by their low photostability or light fastness and they tend to darken and become dull.[18,38] Light fastness is the degree to which a dye resists fading due to light exposure.[39] It is believed that the chromophore determines the general light fastness properties of a dye, while the auxochrome can alter the light fastness properties such as improving the light fastness if in the *meta* position;[18,40,41] this position and other positions of functional groups are shown in Figure 1.2.

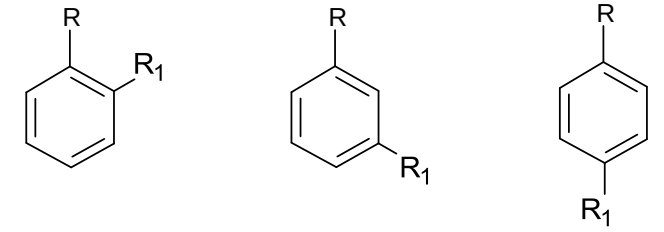


Figure 1.2 Positions of Functional Groups

1.2.1.2. Solvents

Solvents are used as vehicles to carry the dyes through the ballpoint pen system and to facilitate its application onto the paper.[25] Ink formulations originally used oil-based vehicles, such as olein, but changed to glycol-based vehicles.[23,26] This was to improve drying time and make use of dyes that are soluble in glycol which outnumber those that are soluble in oil. Typically solvents are non-volatile, such as 1,2-propylene glycol, phenoxyethanol and butylene glycol.[42]

As stated before, the composition of inks is dependent on the manufacturer and one example is the relative content of phenoxyethanol which varies considerably

among different ballpoint pens.[42] Research has also shown that 95% of all ballpoint inks contain phenoxyethanol as a major solvent.[43] Figure 1.3 shows the chemical structures of some solvents used in ballpoint pen ink. Phthalic anhydride is also included, as it will form non-volatile liquid phthalates with most of the solvent components on drying.

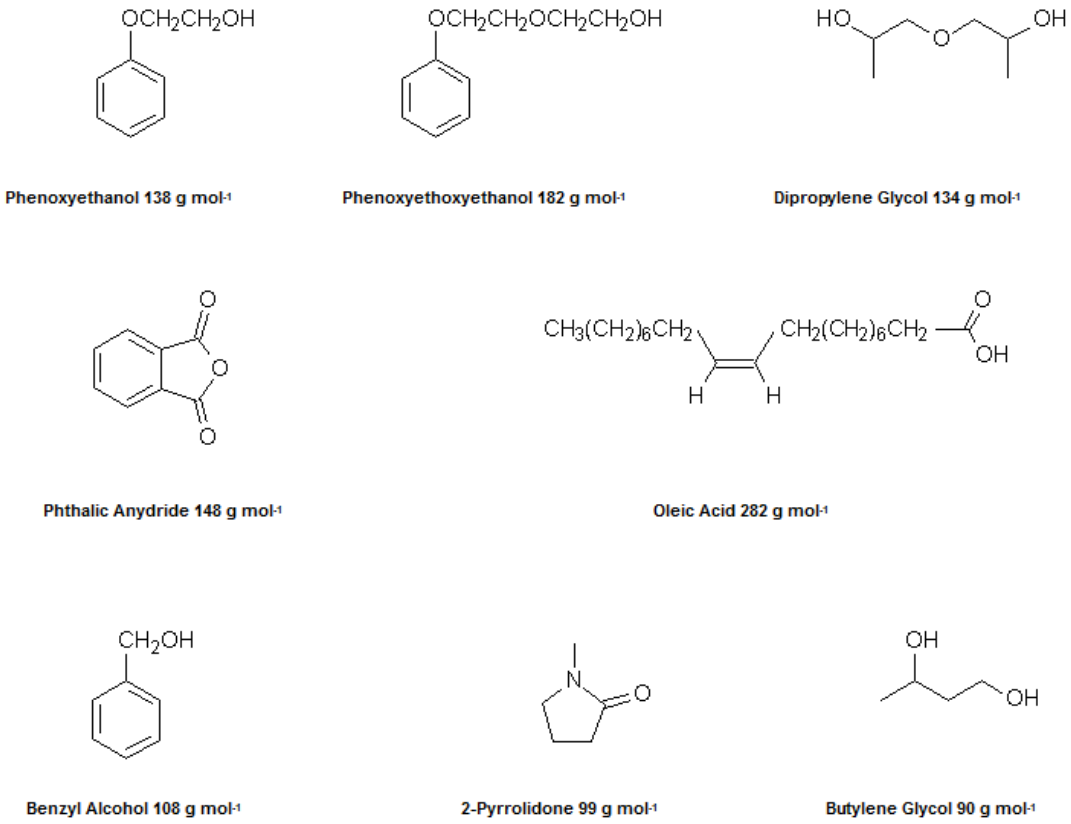
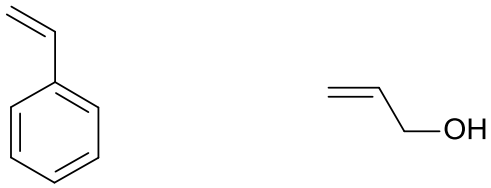


Figure 1.3 Structures of Solvents found in Ballpoint Pen Inks[43]

1.2.1.3 Resins

Resins are used for a number of reasons such as altering the lubricant qualities of the ink,[44] to extend the ink (filler) and to thicken the ink, making it more viscous.[28,45] Additionally, when resins dry they form a chemical bond between the ink and the paper which makes removal of the ink harder. Figure 1.4 gives examples of resin monomers used in ballpoint pen ink.



Styrene **Allyl Alcohol**

Figure 1.4 Structures of Resins found in Ballpoint Pen Ink[44]

1.3 Ink Aging

The substances present in ink are all subject to aging of various types including; drying through solvent evaporation, loss of colour through oxidation of the dye and hardening due to resin polymerisation.[26,46,47] It is a combination of these processes that causes ink degradation and therefore ink fading. It has been found that ink in a cartridge undergoes little, if any degradation,[48] it is once the ink is applied to the paper that the processes begin. These aging processes have been studied and much research has been carried out into the evaporation of solvents.[11,21,49]

The system consisting of ink on a paper substrate is a complex one. When ink is placed on paper, it lies on the surface and also penetrates into the paper. The extent to which depends on both the individual ink and on the paper substrate. The primary function of the vehicle is to aid the application of the ink onto the substrate, once applied, the ink needs to dry and the main process is through simple solvent evaporation[44,45] from the surface of the ink line. To reach the surface in order to evaporate, the vehicle must diffuse from within the ink layer. However, resins and other ballpoint ink ingredients may limit the diffusion process due to cross-linking or polymerisation. The diffusion process slows and at a certain stage it stops completely and the remaining volatile components of the ink will remain inside the aging ink line.[21] Solvent vapours not only escape from the surface of the ink, but also diffuse through the paper laterally and downwardly. It has been shown that ballpoint ink solvents can be found bordering the ink line (lateral diffusion), on paper that lies on top of the ink (atmospheric evaporation) and on paper that was under the document (diffusion through the paper and

evaporation from the opposite side).[45] It is also possible for the colorants to remain fixed on the paper fibres but this depends on the affinity of the colorants to the cellulose fibres.[49]

Paper also has an important role in the aging of inks. In practice, it is impossible to ensure the homogeneity of the ink applied on paper. The influence of substrate structure (paper type) on the drying process should not be underestimated, as the porosity can differ widely within the same sheet of paper.[42] Paper properties, especially surface and capillary structure, may play a significant role in the drying process.[4,5] For paper, the process of aging starts after its manufacture, whereas for ink, the process generally begins after it is applied to the paper. After their manufacture, inks are stored in containers (pen cartridges and ink bottles) classed as a closed system and their aging in these containers is negligible compared with the aging which starts as soon as ink is deposited on paper (an open system).[25,28,46] However, changes to the dye components of the ink may start as soon as they are exposed to light which leads to degradation, (photofading). As a consequence of the aging process of cellulose, oxidised groups, which correspond to carbonyl and carboxyl groups, are formed on the cellulose chains via a process called hydrolytic chain scission.[13,50] As stated previously, ink formulations are unique chemical mixtures and the aging process is inherently formula dependent, however the aging of the substrate will also contribute to the degradation process and both are influenced by external and environmental factors such as temperature, light and (singlet) oxygen.[5]

1.3.1 Dye Degradation

Dyes are most often used as the component for discrimination between different ballpoint pen inks. [13,15,16,18,51-54] While the solvents and other organic components volatilise, the dyes are non-volatile and remain on the paper.[25] As mentioned previously in section 1.2.1.1, the light fastness of a dye is defined as its ability to resist fading by light exposure. Dyes typically used in ballpoint inks are chosen for their strong colour, but this does not last. The dyes fade and this is dependent on the structure, but also external factors. The type of light or light source also has an effect and research has shown that dyes degrade ten times faster in

artificial light than sunlight[55] and fading is faster with ultraviolet light than with visible light.[56] If the dye is in solution, this has been shown to have an effect on the rate of degradation also.[57]

Dyes are not just used in inks, but in numerous industries, including textiles, paper, cosmetics and food to name a few.[30,33,58] The chemistry of dyes, their analysis and degradation has wide reaching implications not just within forensic science but also environmentally.[26,29,59] Dye degradation is an important issue as some dyes produce toxic products[60] and research using photodegradation and biodegradation methods has been used to find ways to clean and discolour textile wastewater.[61-65]

During photofading, triphenylmethane dyes undergo a photochemical reaction that begins with the absorption of visible or ultraviolet light. Absorption of a photon by an organic molecule leads to the formation of an electronically excited state. This is then the starting point for further reaction steps. More than one pathway is possible and subsequently many products can be formed.

The degradation of triphenylmethane dyes is characterised by demethylation whereby successive methyl groups (-CH_3) present in the aromatic structure are replaced by hydrogen atoms (H), resulting in a net loss of 14 mass units. The products produced are known as the dye homologues. The demethylation mechanism is summarised in Figure 1.5.

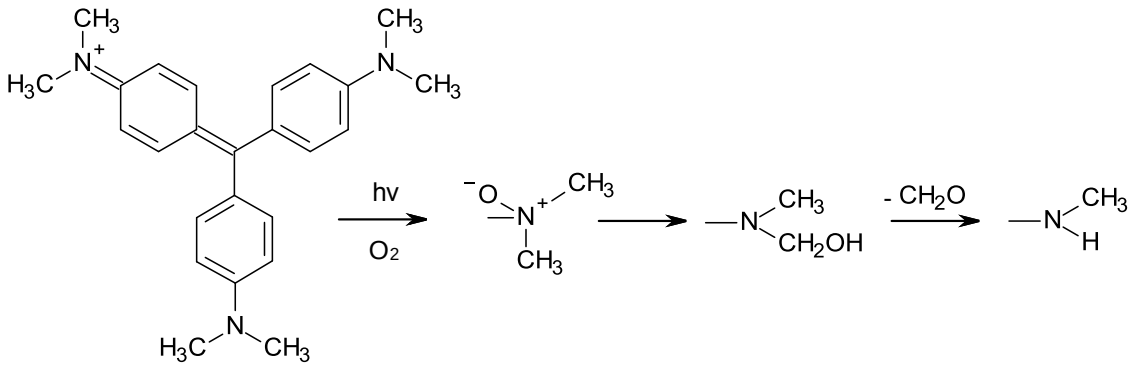


Figure 1.5 Mechanism of Demethylation of a Triphenylmethane dye

This process is not light dependent, dyes will degrade naturally in the dark, but the process is much slower.[5,59] Another pathway is photo-oxidative cleavage of the central conjugated structure. This leads to the production of benzophenones and phenols as shown in Figure 1.6.[66,67] Natural sunlight (h_v) has been shown to induce oxidation of Basic Violet 3 to form 4-dimethylaminobenzophenone.[68] This is likely to occur via singlet oxygen as triarylmethane dyes can produce singlet oxygen upon photolysis on paper.[57] Singlet oxygen is produced by the dye absorbing light and the excited singlet state of the dye converts to a triplet state by inter system crossing. The triplet state can react with ordinary triplet O_2 giving the single state of the dye and producing triplet oxygen.

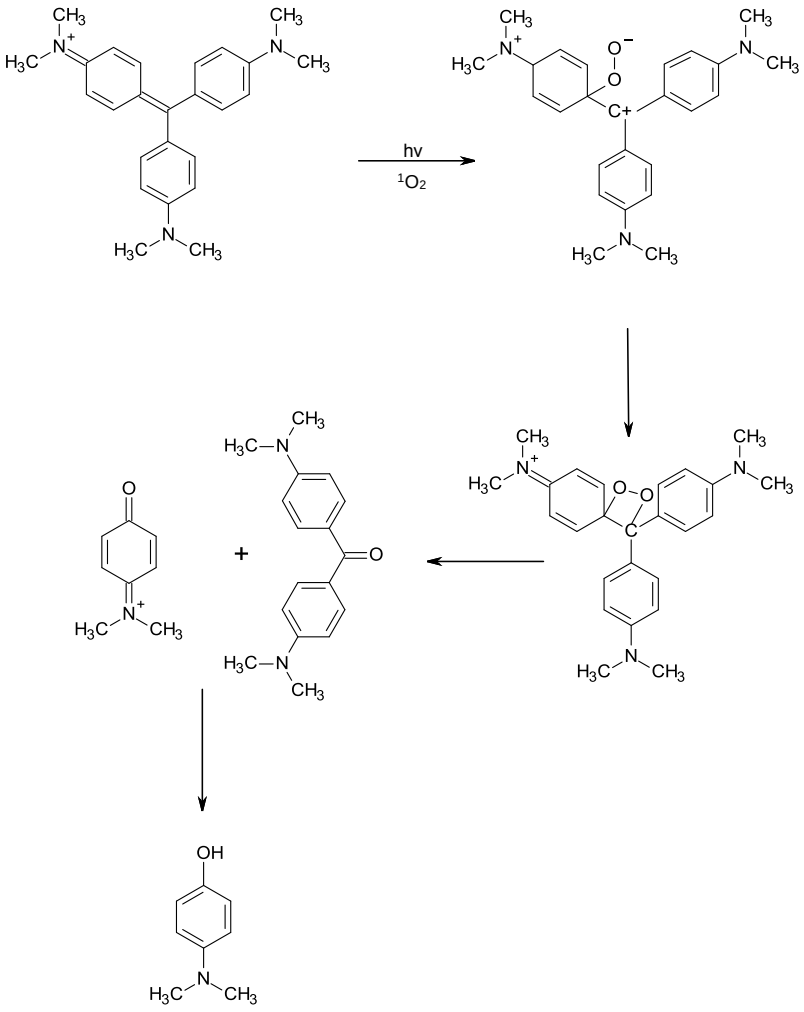


Figure 1.6 Oxidative Cleavage of Basic Violet 3 by Singlet Oxygen

In section 1.3, external factors were discussed that may also influence the process. The pathways can be affected by the substrate and solvent as they may have the ability to donate electrons or hydrogen atoms to the dyes.[57,59] The pathways can also be photocatalysed by the presence of, for example, titanium dioxide which is a common white pigment used in paper manufacturing, but can also act as a singlet oxygen sensitiser.[69,70] A sensitiser facilitates the start of a reaction, in this case titanium dioxide can produce singlet oxygen when irradiated. Both the pathways discussed may occur under the same conditions, which puts them in competition with each other.[61]

1.3.2 Ink Dating

The examination of writing inks is usually performed for identification, comparison or dating purposes. Dating an ink entry on paper is not straightforward and has been discussed by many authors.[8,10,11,39,41,42,44,71-78] This is mainly due to the great variety of inks that exist, the variety of chemical processes that inks undergo from the time they are deposited onto the substrate and the unknown and uncontrolled external factors that can influence these processes.[75] Age determination of inks dates back to the 1930's when chemical tests were used on inks that contained the pigment iron gallotannate, specifically fountain pen inks.[79] The rate of reaction of the ink with oxalic acid allowed estimations to be made of the age, based on the time since it was deposited on the paper. The rate of bleaching of freshly written inks was much higher than that of inks that were several years old.[21] Chemical tests are destructive and are rarely used today.

Two fundamental approaches to ink dating have been established; the static approach and the dynamic approach.

1.3.2.1 Static Dating

The static approach to ink dating involves establishing a profile that reflects the composition of the ink.[75] The 'profile' consists of the optical properties and a thin-layer chromatogram of the inks. A library would contain the profiles of a number

of different ink samples. Dating by this method allows differentiation of inks based on any differences in the profiles, the identification of the source of an ink by matching its profile to one in a library and the date of an ink's first production. In addition to the composition, fluorescent tags have been added to ink formulations by some manufacturers to indicate the year in which an ink was brought onto the market. These samples were part of an ink tagging program that existed in the United States between 1970 and 1994 and helped with backdating documents.[11] The US Secret Service are currently collaborating with manufacturers to encourage the addition of tags to their ink products.[11]

There are however limitations to using the static approach. Collaboration between the manufacturers and custodians of the library is required in order to keep the information up-to-date. Also, inks undergo chemical processes as they age, so the composition of an ink will change over time which needs to be considered when comparing profiles.

An ink library does currently exist and is maintained by the US Secret Service. The reference collection contains more than 10 800 domestic and international writing ink samples, some of which date back to the 1800's.[8] This library will however be far from complete. There is currently no law forcing manufacturers to participate in either an ink tagging programme or an ink library, so there is no way to guarantee that the library contains every ink formula ever created. Despite the limitations, the static method can still be useful for authenticating documents.

1.3.2.2 Dynamic Dating

Initially the dating of inks was limited to a historical timeline when changes were made in the compositions of inks,[54] also known as backdating, but another approach has been developed - dynamic dating, whereby the processes that occur following the deposition of the ink onto the substrate are measured, against time, to produce aging curves. Relative age refers to the comparison of inks which have been treated and stored identically and the only difference is the time when the ink was applied to the substrate. By subjecting the ink sample to heat, light, or water, artificial aging is achieved. The sample is analysed before and after

artificial aging, allowing the production of an aging curve.[80] Age determinations are based upon the idea that the longer an ink has been on a document, the more physical and chemical changes occur. If these changes can be measured and related to the aging process for known dated entries of the same formulation, it is possible to determine the relative age of the questioned entry. The changes which an ink formulation undergoes during the aging process include evaporation of solvents, oxidation and/or polymerisation of resins and degradation of colourants.[75]

The hardening of resins after the deposition of inks on the paper begins immediately.[75] One of the purposes of resins is to help bind the ink to the paper. This is achieved by polymerisation between the dyes and the resins. Research has been carried out into the extractability of ink using a solvent and was based on the principle that, the harder it is to extract the ink from the paper, the drier the resin, therefore the older the ink is and *vice versa*. One technique; solvent-extraction demonstrates the relationship between the age of a ballpoint ink and the rate at which the ink can be extracted from paper.[80] Kikuchi provided the basis for the solvent extraction techniques used today.[81] The longer the ink has been on paper, the slower the extraction process. Other methods include R-Ratio which measures the amount of ink extracted after a period of time. *L*th extraction time measures the time it takes to extract a certain amount (*L*) of ink and percentage extraction measures the percentage of the ink that can be extracted before no more can be removed,[79,80,82,83] instead of the rate of ink extraction.

Solvents are classed as volatile ink components. Up to 90% of volatile components will evaporate as soon as the ink is deposited on the paper.[75] Solvent evaporation can be measured and can be used as an indicator for the age of an ink.[84-86] Gas chromatography has been used to analyse inks in terms of aging.[11] The rate of evaporation is considered to be rapid in the first 6-8 months, then levels off up to 18 months, and after 2 years it is considered to have ceased and volatile components are no longer useful for determining age.[87] Phenoxyethanol, as a solvent has been the focus of much research, as it is believed that it is present in 95% of ballpoint pen inks.[88]

The most successful results for ink dating have focussed on the effect of time on the relative solubility of the ink in different solvents, changes in the relative concentrations of dyestuffs and solvents in the ink.[89] With the improvement in analytical techniques, it is now widely accepted that the dating methods based on artificial aging and sequential extraction of dyes, are unreliable[5] due to the inaccuracy of light and heat to mimic natural aging. This has led to the dating of inks and documents being one of the hardest problems facing a document examiner.

All of the above techniques have been successful in ink dating, however, these techniques like many others, rely on the comparison of the questioned ink with standard reference samples, but in many cases, this is not possible. Cantu has reported on the accelerated aging of inks as an estimate of age without access to known-date inks.[86] The evaporation of volatile solvents and the change of extraction efficiencies of dyes into weak solvents, and the change in the ink's infrared spectral characteristics were assessed. These parameters can be measured to produce an aging curve, but the aging curves only refer to inks of the same formula on the same paper.

1.4 Ink Analysis

Inks can be examined using a variety of techniques depending on what property is being tested. Ink lines can be examined using microscopy to determine the type of pen used as different pen types produce distinctive features on the paper. For example, ballpoint pens leave striation marks in the ink line which correspond to the movement of the ball. So although microscopic analysis of ink line morphology is important for determining the class characteristics of ink, other types of chemical analysis are required to differentiate the same type of ink or identify specific inks.

Historically, ink analysis relied upon the use of filters to enhance the contrast between different responses when inks were subjected to light of different wavelengths ranging from ultraviolet to infrared.[54] Chemical spot tests were also used to detect the presence of certain metals in ink pigments for example, iron,

copper, chromium and vanadium.[51] The tests were only partially successful as they were able to determine the type of ink, but they were not able to individualise the inks and therefore could not provide information about ink formulations.[54] With the introduction of ballpoint pens during the 1950's and the difference in the composition of the inks, new techniques had to be developed for the analysis of inks.

The first chromatographic technique to be used to analyse fluid inks was paper chromatography,[90] and it was used to analyse fountain pen inks, stamp inks and ballpoint pen ink.[91] Brown and Kirk used their knowledge of paper chromatography and developed the technique of paper electrophoresis to analyse specifically ballpoint inks,[92] but the technique has also been successfully used to separate felt-tipped pen inks.[93] There were advantages to electrophoresis over chromatography including that dye components that could not be separated by paper chromatography could be separated by electrophoresis, it was quicker and had good reproducibility, however it required a large sample size and was a slow and expensive technique.[93]

Godown, in 1951, was the first person to suggest the use of thin layer chromatography (TLC) for the analysis of fluid inks.[94] A similar technique was applied to ballpoint inks and was shown to be effective for the separation of dye mixtures[44,53,54,95,96] and resulted in TLC effectively replacing paper chromatography from the 1960's because of its superior resolution.[91] Since then TLC has undergone many developments. Brunelle worked on optimising solvent systems and suggested the use of ethyl acetate, ethanol and water (70:35:30) and *n*-butanol, ethanol and water, (50:10:15).[54] Cantu and Kelly proposed a standard method using silica stationary phases and different solvent systems; *n*-butanol, *iso*-propanol, water (2:1:1) and *n*-butanol, ethanol, 10% oxalic acid (50:10:15).[53] Tappolet carried out work on different colours of fountain, ballpoint and fibre tip pens and reported the optimised conditions.[97] Other developments have included the introduction of high performance thin layer chromatography (HPTLC), whereby the particles on the stationary phase plates are smaller than on standard TLC plates.[98] Advantages of HPTLC over TLC include quicker development of the plates, increased sensitivity and increased separation with greater reproducibility.[51] Another development was improved sampling

techniques which led to smaller sample sizes being needed for analysis.[99] In terms of ink analysis in a forensic science context, this is extremely important, in order to minimise the damage done to a document. Up to this point, TLC plates had only been observed under normal lighting conditions, but research carried out suggested the use of infrared luminescence to aid in the visualisation of the plates[100] as some non-visible components may exhibit infrared luminescence. Current methods for ink analysis using TLC have not changed considerably, one of the recommended solvent systems for ballpoint ink is still ethyl acetate, ethanol and water (70:35:50) as suggested by Brunelle, but technology has improved and TLC can now be carried out using a more automated system.[101] TLC as a technique is mainly used for qualitative analysis; quantitative analysis is also possible, but a calibrated densitometer is required.

High performance liquid chromatography (HPLC) is a technique that can be used effectively to analyse the non-coloured components of a mixture as well as coloured components. It was first used successfully to distinguish ballpoint pen inks in the 1970's, based on their dye components.[31] A normal phase system was used in this instance, but subsequent research has used reverse phase systems.[102] HPLC works in a similar way to TLC, but a detector is coupled to the chromatography system to identify components as they elute from the column. Many types of detector are available such as fluorescence and refractive index, but for ink analysis a UV detector has been the most commonly used. It is usual now for photo diode array detectors to be used as they can measure multiple wavelengths and obtain a full UV-visible spectrum of the components, allowing better identification and quantification.[103-105] HPLC also has a better resolving power than TLC and it has been found to be capable of distinguishing inks that were indistinguishable using TLC.[102] As mentioned chromatography is good for qualitative analysis, but HPLC is also capable of quantitative analysis. Measuring the absorbance ratios of resultant peaks in a chromatogram is a method commonly used.[29,106-109]

High performance liquid chromatography has also been used to analyse the dye content of inks and how they change over time under different conditions.[18,52,89,102] Andrasko found that prolonged light exposure resulted in an increase in the relative concentration of the decomposition products,[18] and

compared with compositional changes on exposure to light, the changes due to aging in darkness were much slower.[52] Another study also showed that dye concentrations decrease over time.[89] Application of HPLC for the analysis of ink is not limited to ballpoint ink, it has also been used to analyse printing inks,[103] red ink from seals[76] and non-ball pen ink.[104] In all cases, HPLC has been successful in distinguishing between samples.

Lighting techniques have also been established for the use in ink analysis; von Bremen carried out research on blue inks using ultra-violet (UV) fluorescence as a means of ink differentiation.[110] Dichroic filters were suggested as part of a preliminary ink examination, however a large number of filters would be needed.[111,112] Infrared (IR) luminescence of writing inks has also been successful for the examination of obliterations and alterations[113] and is thought to be the most useful non-destructive method for ink examination.[114] Filtered light examination, using IR and UV light to examine IR luminescence, IR reflectance and UV fluorescence is commonly used today, as it is a simple method for ink examination, it is non-destructive, relatively cheap and easy to use, however, it does not allow for the identification of the ink.[115,116]

Spectroscopic methods that have been used for ink analysis include infrared spectroscopy and Fourier Transform Infrared Spectroscopy (FTIR). Initial research was conducted on photocopy toners and printing inks where good discrimination between samples was achieved,[117] but ballpoint inks were also studied and the first ink dating tests using this technique were completed.[118] Changes in the hydroxyl (-OH) and carbonyl (C=O) absorption bands were observed as the ink aged and also the absorption bands of resins decreased faster than the bands for the dyes in the ink. Recent studies have looked at the use of micro FTIR and Attenuated Total Reflectance FTIR for the examination and discrimination of inks[119] and line crossings[120] with a high degree of success.

A current method used for the non-destructive testing of samples is Raman Spectroscopy. It has been utilised for a range of samples such as the examination of paints,[121] fibres,[122] explosives,[123] drugs,[124] and inks. A laser is focussed on the sample which causes scattering of the light by the sample which is characteristic of chemical structures. Claybourn was one of the first to use Raman spectroscopy

for ink analysis and was able to distinguish between chemically different black ballpoint inks.[125] A development in the technique led to Surface Enhanced Resonance Raman Spectroscopy (SERRS), whereby a colloid is applied to the surface to enhance the Raman signal and the wavelength of the excitation laser can be changed. This method has been used to examine dyes in a variety of samples such as lipstick, shoe polish and inks.[126] It was able to distinguish blue and black ballpoint ink,[127,128] as well as coloured fluid inks[126] and gel inks.[129] A potential issue with SERRS is the long term implications of applying colloid to samples, but studies have shown that colloid treated samples remain stable and still produce SERRS spectra after many years in storage and the spectra are comparable with the original spectra.[130]

Mass spectrometry (MS) can be used to examine a whole host of sample types and it has been coupled with traditional chromatography techniques such as gas and HPLC to analyse inks.[54] Dyes in food and wastewater have been identified using thermospray ionisation mass spectrometry from a health and environmental point of view for a number of years,[131,132] but no literature has been found where it has specifically been used for ink analysis. Field desorption mass spectrometry (FDMS) is well suited to the analysis of non-volatilised compounds such as dyes and research has shown that FDMS is a simple and rapid method for analysing ballpoint pen inks and is able to distinguish inks based on the dyes present.[133] Laser desorption (LD) and fast atom bombardment (FAB) can provide molecular level information concerning an ink's composition and direct analysis of ink on paper has been carried out,[36,69] as well as quantitative analysis of dye classes.[134] Results showed that both techniques were suitable for ink analysis, however LD was more effective for aging studies as it was able to characterise the degradation products.[26] LD is limited to detecting dyes that are neutral or singly charged, but by using matrix assisted LDMS, multiply charged dyes that are used in modern inks have been detected.[135] Electrospray ionisation (ESI) is also a technique that is being increasingly utilised to examine inks and dyes. Blue, black and red ballpoint pen inks were compared based on their mass spectra following ESI-MS.[32,136] Each dye produced a characteristic ion peak and in combination with other dyes present, a dye profile was created which enabled discrimination. ESI-MS has also been used to characterise dyes and identify intermediates formed during degradation.[32,59,137,138] Atmospheric Pressure

Chemical Ionisation (APCI) is another similar ionisation technique which has been used to examine dyes, but papers using this technique are limited[32,139] and no papers have been found where it is has been specifically used to analyse ink.

There has been much empirical research conducted in the area of ink analysis and the aging process as mentioned previously. Numerous papers have been published concerning the chemical processes involved in the fading of dyes in solution by light,[18,59] heat[118] and chemical bleaching.[140] However, little work has been done on the mechanisms followed when such processes occur on physical substrates such as paper[66] and in most cases no products have been identified fully. In addition all of the dating methods rely on artificial aging using the same pen and comparison to reference samples. This is only accurate if the original pen is available, details of storage conditions of the document and the parameters mentioned previously are known. In casework this is rarely the case.

1.5 Instrumental

1.5.1 Filtered Light Examination

Filtered Light Examination (FLE) is the term used to describe the examination of ink and paper using a range of light sources and filters. Observations are made of the UV fluorescence, IR reflectance and IR luminescence of the samples. These can be achieved using separate pieces of apparatus, but more commonly now, instrumentation specially designed to incorporate all three light sources is used, such as the VSC 5000 made by Foster and Freeman Ltd. FLE is one of the easiest and least expensive techniques to perform and is non-destructive. Consequently, it is usually performed first in a questioned document examination before more complex and destructive techniques. The IR radiation used falls in the range 700 – 1000 nm. IR reflectance analysis simply monitors the degree to which the incident light is reflected. Filters are used so that reflectance can be observed at different wavelengths.

Fluorescence is a type of luminescence, however, in this context, by convention, UV fluorescence refers to emissions stimulated by radiation in the UV region and

observed in the visible region, whereas, IR luminescence refers to fluorescence stimulated in the visible or near-infrared (NIR) region and emitted in the IR region.

The phenomenon of fluorescence is caused by excitation of molecules following absorption of electromagnetic radiation. Energy of a given wavelength is absorbed by a molecule and is emitted effectively instantaneously at a longer wavelength, returning the molecule to its ground state, see Figure 1.7. Absorption can cause excitation of the analyte to state 1 or state 2. Once excited, the excess energy can be lost by emission of a photon (shown as solid lines) or by nonradiative processes (dashed line). The duration of fluorescence is approximately 10^{-8} s and effectively ceases when the excitation source is removed.[141] Emissions can be viewed either directly, for the visible part of the spectrum, or indirectly, by way of an image capture system. The occurrence of luminescence or fluorescence depends on the chemicals present in the sample, which allows differentiation of inks by virtue of the variation in composition.

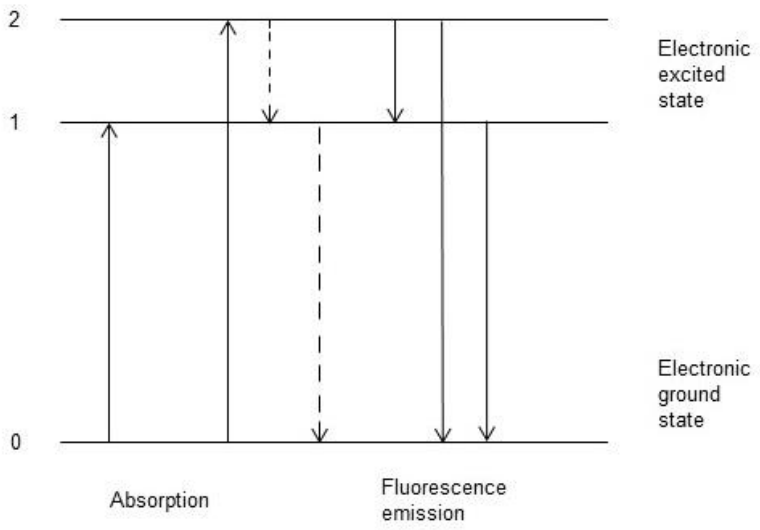


Figure 1.7 Energy Level diagram showing Fluorescence Emission

The UV region of the electromagnetic spectrum covers the range 200 – 400 nm. Excitation is usually carried out at a standard long wavelength, e.g. 366 nm and short wavelength, e.g. 254 nm.

IR luminescence is stimulated by wavelengths in the region of 450 nm. The efficiency of IR luminescence is low, which means that a high intensity source is often necessary to produce a detectable response. Lasers have been employed

as a radiation source for this reason. They are also used because they preclude the use of filters between the source and the sample due to their monochromatic nature. The variation and intensity of IR luminescence for inks is much greater than that for UV fluorescence and therefore, it is often of greater use to the document examiner.

1.5.2 Mass Spectrometry

Mass spectrometry (MS) is an analytical technique that detects samples based on their mass-to-charge ratio *(m/z).* It is a technique that was first used in the early 20th century and work focussed on the analysis of isotopes.[142] In MS, analyte molecules are converted to ions by bombarding them with high-energy electrons. The ions which are formed are then separated based on their mass-to-charge ratio and then quantified through conversion to an electrical signal to produce a mass spectrum of abundance versus *m/z.* Mass spectrometry tends to focus on volatile organic molecules and can be coupled with chromatographic techniques such as gas chromatography and liquid chromatography.

1.5.2.1 Mass Spectrometer Components

Mass spectrometry can be used for the analysis of solids, liquids and gases. The sample enters the system through the inlet system, which introduces the sample to the ionisation chamber where the molecules are converted into gaseous ions by bombardment with electrons, photons, ions or molecules.

1.5.2.2 Ion Sources

Ionisation techniques are often classified as soft ionisation, where little or no fragmentation occurs and hard ionisation where fragmentation is extensive. Table 1.3 lists many of the ionisation techniques that can be used in mass spectrometry.

For gas-phase MS, the sample is vaporised, then ionised. In desorption, the sample is converted directly to gaseous ions. The most commonly used ionisation

technique is Electron Impact (EI) whereby the molecules are bombarded with a high-energy beam of electrons, which produces the ions. It is used typically with gas chromatography.

Table 1.3 Common Sources for Mass Spectrometry[143]

Basic Type	Name and Acronym	Method of Ionisation	Type of Spectra
Gas-phase	Electron Impact (EI)	Energetic electrons	Fragmentation Patterns
	Chemical Ionisation (CI)	Reagent gaseous ions	Proton adducts, few fragments
	Atmospheric Pressure Chemical ionisation (APCI)	Energetic electrons	Singly charged ions, few fragments
Desorption	Fast Atom Bombardment (FAB)	Energetic atomic beam	Molecular ions and fragments
	Matrix Assisted Laser Desorption/Ionisation (MALDI)	High-energy photons	Molecular ions, multiply charged ions
	Electrospray Ionisation (ESI)	Electric field produces charged spray which desolvates	Multiply charged molecular ions

For Electrospray Ionisation (ESI), an electric field is applied to the liquid which causes it to form an aerosol. Ions escape from the aerosol droplets and are detected by a mass analyser. Chemical ionisation (CI) allows the formation of protonated molecules via gas-phase under vacuum. Atmospheric Pressure Chemical Ionisation (APCI) works on the same principles as CI, but takes place at atmospheric pressure instead of under vacuum. Like with ESI, an aerosol is formed from the liquid sample. The droplets then travel through a vaporiser tube where they are heated and become vapour. A high voltage creates a corona discharge that forms electrons which ionise the sample molecules. The analytes proceed to travel to the ion analyser and are detected by an electron multiplier detector.

1.5.2.3 Mass Analysers

Mass analysers are required to distinguish minute mass differences across a large range, but this is not always possible. There are various analysers available which work in different ways (as shown in Table 1.4) and which satisfy different conditions. Time-of-flight analysers can detect a large mass range, but have a low sensitivity and resolution, whereas magnetic sector analyses have a high resolution.

Table 1.4 Common Mass Analysers for Mass Spectrometry[143]

Basic Type	**Analysis Principle**
Magnetic Sector	Deflection of ions in a magnetic field. Ion trajectories depend on *m/z* value.
Double Focusing	Electrostatic focusing followed by magnetic field deflection. Trajectories depend on *m/z* values.
Quadrupole	Ion motion in dc and radio-frequency fields. Only certain *m/z* values are passed.
Ion Trap	Storage of ions in space defined by ring and end cap electrodes. Electric field sequentially ejects ions of increasing *m/z* values.
Ion Cyclotron Resonance	Trapping of ions in cubic cell under influence of trapping voltage and magnetic field. Orbital frequency related inversely to *m/z* value.
Time-of-flight	Equal kinetic energy ions enter drift tube. Drift velocity and thus arrival time at detector depend on mass.

Recently, interest in using mass spectrometry to analyse inks has increased and various ionisation techniques coupled with mass spectrometry have been used.[144]

1.5.3 Atmospheric Pressure Chemical Ionisation

In APCI the liquid sample enters the system and is surrounded by inert gas in a heated region. The combination of gas and heat forms an aerosol from the mobile phase eluent, which starts to evaporate. A corona discharge pin with a high potential is placed near the heated region and produces an electrical discharge which ionises molecules within the aerosol leading to the formation of a gas plasma. When the analyte molecules enter the gas plasma, they can be ionised

via transfer of protons to give a positive or negative ion which then enter the mass spectrometer for analysis as shown in Figure 1.8.

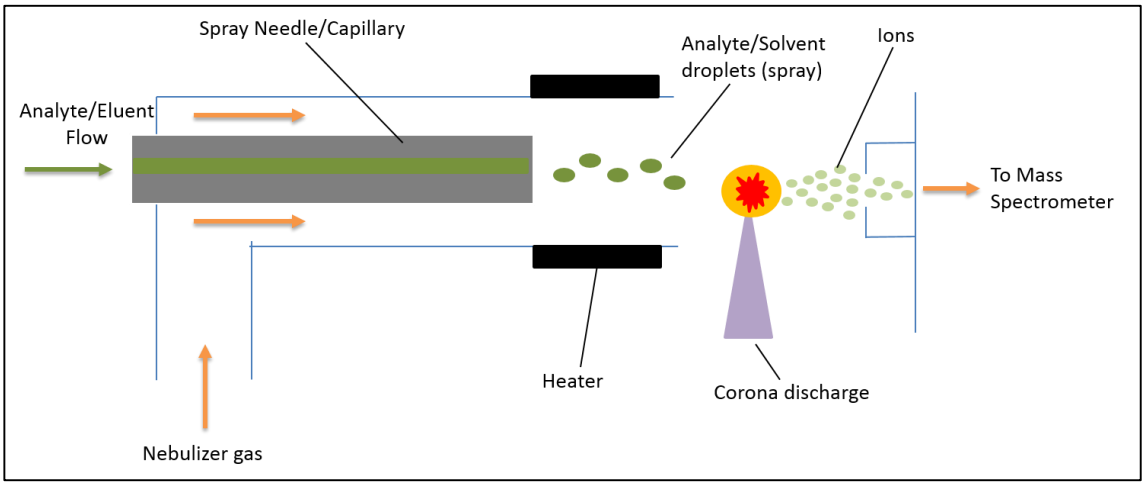


Figure 1.8 Schematic of APCI system

Atmospheric pressure ionisation techniques have been shown to have advantages over other techniques such as easy coupling to liquid separation techniques, better sensitivity, signal stability and reproducibility.[139] APCI is less time consuming than chromatographic approaches since separation of components by time is not required. Also, unlike elution or migration times that may change over time, mass-to-charge ratio measurements are absolute values.[32] APCI with mass spectrometry is becoming a powerful technique for the identification of dyes, it is a soft ionisation technique that can provide molecular information and as a result yield a mass spectrum of component dyes in ink. The APCI-MS system used is shown in Figure 1.9.

Figure 1.9 Photograph of APCI-MS equipment used

As detailed above the majority of methods for determining the degree of aging rely on the identification and relative amounts of coloured degradation products, but none of these are appropriate if the ink has faded completely. Inks do fade and the rate depends on numerous conditions, but eventually many inks will completely lose their colour. In certain circumstances the ink may have been deliberately faded, using sodium hypochlorite bleach or hydrogen peroxide, to alter the content of a document. Common forgery includes modifications of information, such as dates or values. The nature of dyes make them valuable to the forensic document examiner because the dyes can remain on the paper in the form of letters, marks or lines after the solvent has evaporated. Little work has been carried out looking at whether the faded ink entries would still provide useful information and no research has been found that discusses the possibility of using the faded entries for any kind of analysis. It would be beneficial to determine if these faded entries have an importance in ink analysis. This is a novel approach to document examination as no research of this nature has been carried out.

It is believed that the information gained from this project would be extremely valuable for the forensic science community and historical organisations, for the examination of writing on documents where the ink has faded either naturally over a period of time, or where the ink has been affected by other conditions such as light, heat or chemical bleaching.

1.6 Aims

A problem that can be encountered by forensic document examiners is the detection and rectification of document fraud where, for example, new signatures or values have been added to documents after the originals have faded following exposure to light or bleaching agents.

The objectives of this project are to:

- Examine a range of ballpoint pen inks of different colours, representative of those available in the UK using Atmospheric Pressure Chemical Ionisation Mass Spectrometry
- Investigate the chemical processes that occur and the ultimate fading products that are formed after the deposition of ink onto a solid substrate and when subjected to light, hydrogen peroxide and sodium hypochlorite bleach
- Investigate the aging processes of dye standards in solution when subjected to light, hydrogen peroxide and chlorine bleach
- Investigate the aging process of dye mixtures in solution when subjected to light, hydrogen peroxide and chlorine bleach

In assessing the objectives listed above, the main aim of this study is to analyse in detail mass spectrometry results from APCI to further understand the aging process of inks and dyes when faded deliberately by light, bleach and peroxide.

Chapter 2 Experimental Techniques

2.1 Sampling

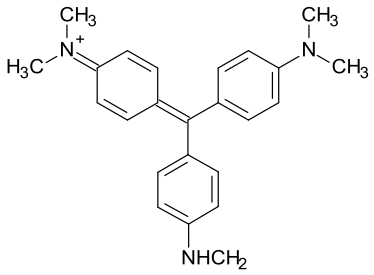
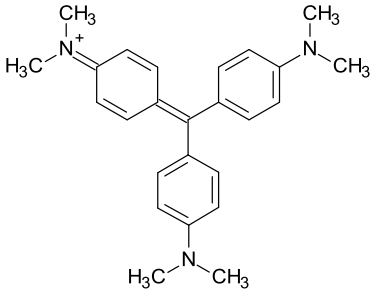
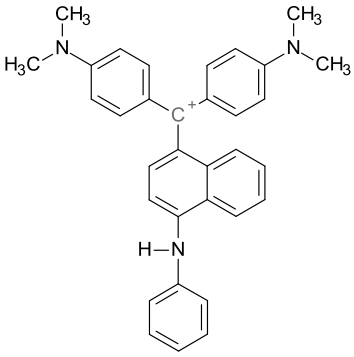
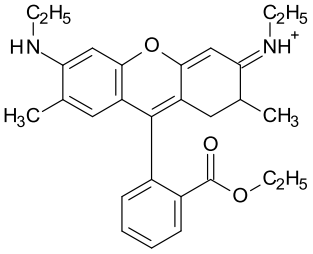
As ballpoint pens are still the most common type of pen used to create documents, they have been chosen for analysis in this project. 19 ballpoint pens were purchased from high street stores to provide a random sample set that was representative of those in use in the UK today and that could be encountered as part of a forensic document examination. Table 2.1 shows the identification and colour of the samples. The pens were stored in cool, dark conditions when not in use.

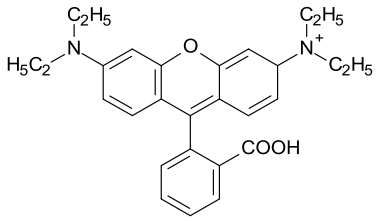
Table 2.1 Identification of Pen Samples

Number	Colour	Brand
1	Blue	Micron™
2	Blue	Bic™
3	Blue	Staedtler™
4	Blue	Woolworths™
5	Black	Micron™
6	Black	Bic™
7	Black	Staedtler™
8	Black	Woolworths™
9	Red	Micron™
10	Red	Bic™ ™
11	Red	Staedtler
12	Red	Woolworths ™
13	Green	Bic™
14	Green	Galileo™
15	Black	Papermate Flexigrip™
16	Black	Galileo™
17	Black	Papermate Comfortmate™
18	Green	Micron™
19	Purple	Bic™

In order to understand the processes occurring solely to the dyes in the inks, pure dye standards of common dyes used in inks were obtained from Simpson UK Ltd, Gwent, as listed in Table 2.2.

Table 2.2 Dye Standards

Dye	Other Name(s)	C.I Number	Structure	m/z
Basic Violet 1	Methyl Violet (2B)	42535		358
Basic Violet 3	Crystal Violet, Methyl Violet 10B	42555		372
Basic Blue 26	Victoria Blue B	44045		470
Basic Red 1	Rhodamine 6G	45160		443

Basic Violet 10	Rhodamine 6B	45170		443

Dye standard combinations were also analysed to replicate dye combinations present in ballpoint inks. The combinations used in this study were determined based on APCI-MS results that were carried out on the 19 ballpoint pen samples used. The ratios and the dye combinations corresponding to the pen samples are shown in Table 2.3.

Table 2.3 Dye Standard Combinations

Dye Standard Combination	**Dye standards proportions**	**Pen Colour**	**Pen number**
1	BV1 20 : BV3 15	Blue	2
2	BB26 100 : BV3 50	Blue	1
3	BV1 100 : BV3 70 : BB26 20	Blue	4
4	BV1 100 : BV3 40	Black	5
5	BV3 100 : BV1 40	Black	6
6	BV1 100 : BV3 65	Black	7
7	BV1 100 : BV3 85 : BR1 50	Black	8
8	BR1 100 : BV1 10	Red	11
9	BV3 100 : BV1 100	Black	15
10	BR1 100 : BV10 100	Purple	19

2.1.1 Sample Preparation

Samples were subjected to a range of aging methods to simulate different circumstances that may be encountered in casework. These can be categorised as natural or artificial aging methods.

2.1.1.1 Natural Aging

Each ballpoint pen was used to write approximately 20 cm of written text on white *Antalis Multitude Multifunction paper A4* (80gm^{-2}). Standard office type paper was chosen, as it is a common substrate used to write on. Care was taken to apply normal writing pressure by the same donor and produce comparable line quality. The samples were duplicated. For one set of samples, each sample was placed between two blank pieces of the same type of paper and stored in a cardboard folder at room temperature. This “sandwich effect” was used to prevent migration of solvents from one sample sheet to another. It also helps to prevent contamination from the folder. The duplicate set of samples was attached to the inside of a window facing north-eastwards for exposure to daylight and left for specific lengths of time until analysis. An *EDDI Peak* light meter was used to record the light levels during this time.

2.1.1.2 Artificial Aging

Each ballpoint pen was used to write approximately 20 cm of written text on white *Antalis Multitude Multifunction paper A4* (80gm^{-2}). One set of samples were treated with 5% hydrogen peroxide (*Fisher*). This was applied using a small paintbrush which was wiped gently on the surface of the ink to minimise any damage to the paper. Multiple applications were needed in order to fade the ink. Between each application, the samples were allowed to dry. A further set of samples were treated in the same way with *Domestos* household bleach (containing < 5% chlorine).

Dye standards are in powder form and were prepared by dissolving 0.001 g in 5 mL of Methanol *(Fisher HPLC Grade)* and 995 mL of distilled water. For each dye standard, five 30 mL samples were prepared. One set of samples were placed in a fume cupboard and subjected to artificial daylight by three *Memolux* 240 V, 20 W bulbs and left until analysed.[145] 2 µl of 5% hydrogen peroxide was added to another two sets of each dye. One of these sets was subjected to artificial daylight; the other set was left in a room and exposed to natural light variations

that occur over time, until the samples were colourless. 2 µl of *Domestos* household bleach (containing <5% chlorine) was added to another two sets of dyes. One set was subjected to artificial daylight, the other left in a room with ambient light until analysed.

The dye standard combinations were subjected to artificial aging using light, hydrogen peroxide and sodium hypochlorite bleach as previously described.

The paper samples were then analysed using filtered light examination to look at the infrared absorbance, reflectance and luminescence characteristics of the samples and all samples were analysed using atmospheric pressure chemical ionisation mass spectrometry to identify components and degradation products present in the samples over time.

2.2 Filtered Light Experimental Details

2.2.1 Filtered Light Materials and Methods

The paper samples containing written entries of each ballpoint pen sample were subjected to FLE examination using a Video Spectral Comparator 5000 (*Foster and Freeman Ltd*). The examination comprised of IR absorbance, IR reflectance and IR luminescence. The samples were analysed for IR absorbance and reflectance at wavelengths 530 nm, 690 nm, 714 nm, 725 nm, 778 nm, 825 nm, 856 nm and 1000 nm with the use of flood lighting and long pass filters. The ranges at which the inks absorb and reflect IR radiation were recorded.

For IR luminescence, each sample was subjected to spot lighting with long and short pass filters. The wavelengths at which the inks luminesced were recorded. Fresh samples were analysed, as were aged samples and the results compared.

2.3 APCI – MS Experimental Details

2.3.1 APCI-MS Materials and Methods

A Finnigan LCQ Advantage Max (*ThermoFisher Scientific*) mass spectrometer with APCI adapter was used for analysis of all of the samples in positive mode as most dyes present in ballpoint pen inks are basic or cationic. The spectrometer has a 3D quadrupole ion trap detector. The mobile phase composed of 75% methanol (*Fischer Scientific HPLC Grade*), 24.5% deionised H_2O and 0.5% acetic acid (*BDH*) with a flow rate of 0.2 mL min^{-1}. The instrument was calibrated using caffeine. Samples were scanned from *m/z* 90 to 900 amu. The acquisition time was 1 min.

2.3.2 Ink Samples

A Harris Micropunch was used to cut five plugs (1.5 mm diameter) from the ink line. Micropunches reduce analysis time as they punch out a disc of paper and dispense the sample straight into a vial, improving the accuracy of the extraction technique.[146]

The plugs were placed in 30 µl methanol (*Fisher Scientific HPLC Grade*) and sonicated for ten minutes. A control of methanol was used. 2 µl of the extract was used for APCI-MS analysis in positive mode as shown in Figure 2.1. A paper blank was obtained by removing five plugs of blank paper near the sampling location of the ink. The blank was extracted and analysed in the same manner as the ink samples.

The ink extracts were analysed without chromatographic separation by direct flow injection a total of three times for each analysis to obtain reproducible results.

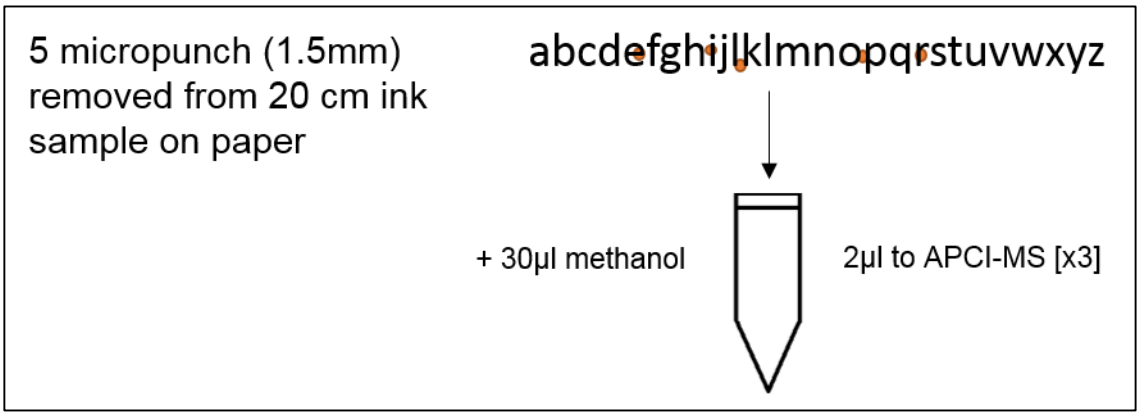


Figure 2.1 Schematic of Sample preparation

The Qual Browser program was used to subtract the total ion chromatogram (TIC) of each paper blank from that of the corresponding ink sample. The final ink profile was obtained by averaging the spectra across the sample peak in the subtracted TIC.

Regular analyses were carried out on samples subjected to daylight and samples stored in darkness over a period of two years. Also samples that were treated with hydrogen peroxide and sodium hypochlorite bleach.

2.3.3 Dye Standards

Dye standards and combinations as shown in Tables 2.2 and 2.3 were analysed directly by flow injection. 2 µl of sample was used for analysis. Samples that had been treated with hydrogen peroxide, sodium hypochlorite bleach and light were also analysed over time as the samples faded.

The Qual Browser program was used to average the spectra across the sample peak in the Total Ion Chromatogram to produce the final dye profile.

Table 2.4 shows the time periods used to analyse the different range of samples.

Table 2.4 Table to show the time periods used to analyse the samples

	Photofading	**Hydrogen Peroxide**	**Sodium Hypochlorite**
Dye Standards	0-6 days	0-6 days	0-6 days
Dye Mixtures	0-10 days	0-10 days	0-10 days
Ink on Paper	0-23 months	0-23 months	0-23 months

Chapter 3 Identification of Ballpoint Ink Dyes

The various dyes present in the ballpoint inks must first be identified so that the sources of the various photochemical or chemical degradation products may be determined. Direct injection Atmospheric Pressure Chemical Ionisation Mass Spectrometry (APCI-MS) was used for the identification as it gave the high sensitivity necessary for the very dilute solutions used and enabled simultaneous identification of the dyestuffs and their degradation products.

3.1 Fresh Dye Standards

Throughout this project, dye standards have been used to assist in the understanding of the aging process and related reactions. The pure dye standards do not contain the same added components that the ink samples do, so information gained from the analysis of these standards alone will help in the understanding of the reactions of the inks. As identified in Table 2.2, five dye standards were analysed. Figure 3.1 shows the mass spectra obtained using APCI-MS of fresh dye samples. The ionisation energy of the mass spectrometer was limited to ensure little fragmentation so that only molecular ions from the dye components should be observed.

Each basic dye produced a positive ion corresponding to the cationic moiety of the dye molecule and Basic Blue 26, Basic Blue 7, Basic Red 1 and Basic Violet 10 all showed nearly pure samples. Basic Blues 26 and 7 contain one strong peak at m/z 470 and 478 respectively. Likewise Basic Red 1 and Basic Violet 10 gave single peaks, but as they are isomers they both have the same m/z of 443, however they can be readily distinguished by their MS/MS spectra. Basic Violet 10 gave a strong daughter ion at m/z 399 due to the loss of CO_2 from the aromatic ring, while Basic Red 1 forms a fragment at m/z 415 after losing an ethene group.

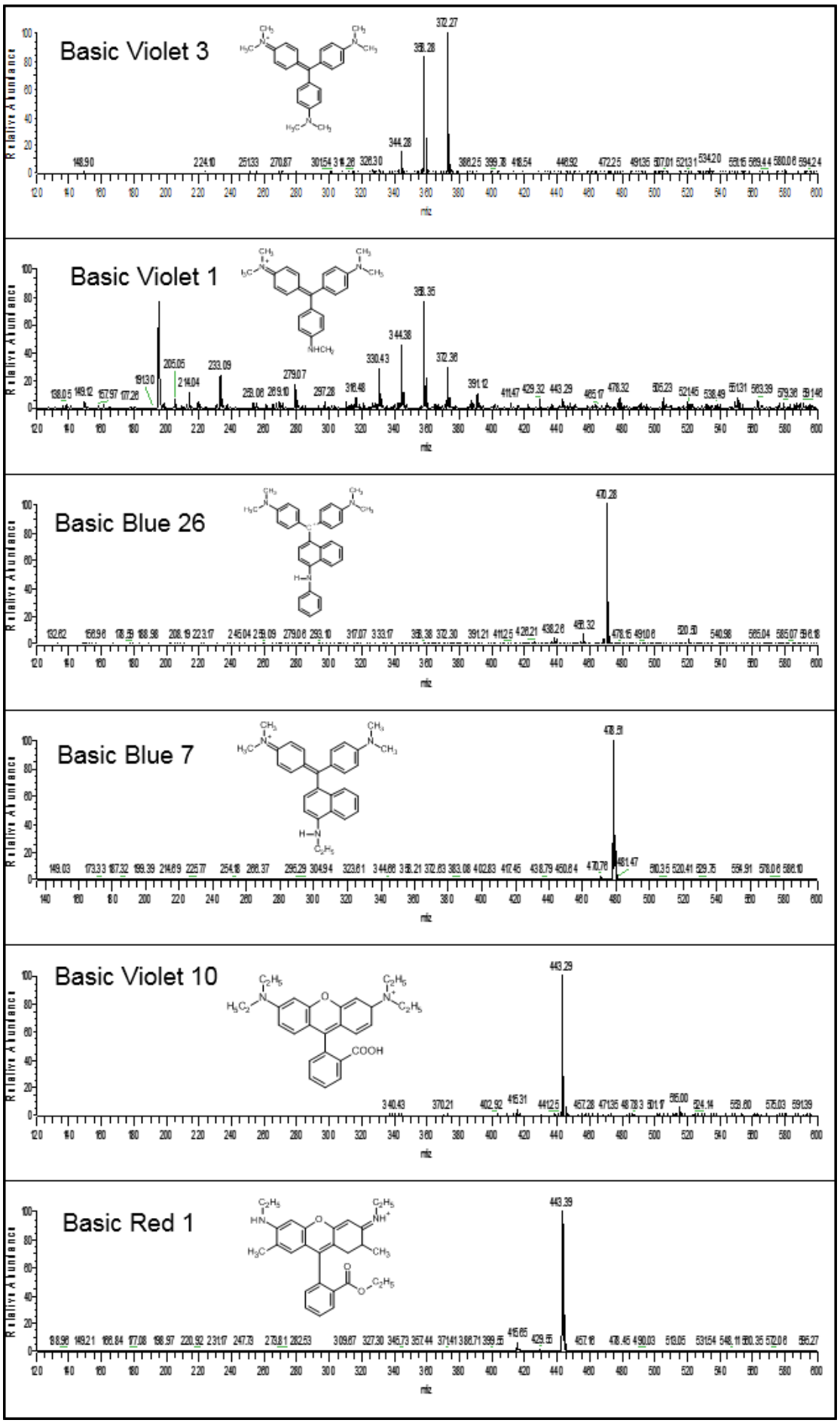


Figure 3.1 APCI-MS Spectra for Dye Standards

It is important to note that for Basic Violet 3 the base peak (largest peak) is 372 m/z, whereas for Basic Violet 1, the base peak is 358 m/z. For each dye standard, the predominant peak corresponds to their stated main component. However additional smaller peaks due to the presence of homologues were observed in the mass spectra of Basic Violet 3 and 1. Additional peaks at m/z 358 and 344 corresponding to the penta-methyl (Basic Violet 1) and tetra-methyl homologues respectively were seen for Basic Violet 3 ($M^{+\bullet}$ m/z 372). The Basic Violet 1 ($M^{+\bullet}$ m/z 358) showed many additional peaks including m/z 372 (Basic Violet 3), 344 (tetra-methyl), 330 (tri-methyl) and 279 and 233 (probable decomposition products). The peak at m/z 195 in the Basic Violet 1 spectrum is not related to the dye, but appears to be a contaminant from the equipment. This shows that both of these fresh standard dye samples contain a mixture of each other. This is not unusual as these dyes are from the same family and are known to be sold as mixtures.[26]

3.2 Fresh Ink Samples

In order to begin to re-visualise faded ink samples, identification of the components and dyes that are present in those samples when they were applied is required. The aging (degradation) processes can then be followed to identify the faded products which may be reacted with a suitable reagent to re-form a coloured product.

Fresh ink entries on paper made by 19 different ballpoint pens as shown in Table 2.1 were analysed by APCI-MS. Although direct injection APCI-MS does not provide chromatographic information, the method does provide a way to obtain information about samples. Using the known m/z values for dyes, identification of some of the dyes and other components present in the ballpoint ink could be made. The results are summarised in Table 3.1.

Table 3.1 Identification of Dyes based on the m/z values present in Ballpoint Pen Samples

Pen	BV 3 372	BV 1 358	BB 26 470	BB 2 484	BB 9 284	BR 1 443	BV 10 443	BG 1 385	BG 4 329	BY 2 268	SO 13 213	Nigrosine 530	Copper Phthalocyanine 575	Aryl Guanidines	Unknown	Total
1	•	•	•													**3**
2	•	•								•					535	**3**
3	•	•	•	•												**4**
4	•	•	•		•			•	•				•			**7**
5	•	•										•				**3**
6	•	•														**2**
7	•	•			•			•	•			•	•			**7**
8	•	•			•	•										**4**
9						•	•									**2**
10						•	•			•						**3**
11						•	•									**2**
12						•	•				•					**3**
13														226,240,254,268		
14															367	
15	•	•			•											**3**
16	•	•														**2**
17	•	•			•										367	**3**
18															367	
19						•				•						**2**
Total /%	**58**	**58**	**16**	**5**	**26**	**31**	**21**	**10**	**10**	**16**	**5**	**16**	**16**			

BV3 – Basic Violet 3, BV1 – Basic Violet 1, BB26- Basic Blue 26, BB2 - Basic Blue 2, BB9 – Basic Blue 9, BR1 – Basic Red 1, BV10 – Basic Violet 10, BG1 – Basic Green 1, BG4 – Basic Green 4 , BY2 – Basic Yellow 2, SO13 – Solvent Orange 13.

The following dyes were detected in the mass spectra of the pens. Basic Violet 1 and 3 were present in 58% of the pens. Basic Red 1 in 31%, Basic Blue 9 was detected in 26% of the pens. Basic Blue 26 and Basic Yellow 2 were each in 16% of the samples. Basic Green 1 and 4 were in 10 % and Solvent Orange 13 and Basic Blue 2 were each present in 5% of pens. There were additional signals at m/z 530 corresponding to nigrosine and m/z 575 for copper phthalocyanine. Also, peaks with masses such as m/z 367 and 516 were detected, but identification could not be made.

The dyes Basic Violet 1 and Basic Violet 3 were present in all blue and black ballpoint inks. Basic Blue 26 was only present in blue pens, but was absent from one blue pen (Bic). Basic Blue 2 was only present in 1 blue pen; Staedtler. Basic Red 1 was present in all red pens and also in 3 black pens and the 1 purple pen. Basic Yellow 2 (m/z 268) was present in the purple pen and in one red pen (Staedtler) and one blue pen (Bic).

Based on the identification of components present using APCI-MS, all of the blue pens had a different composition and could all be distinguished from each other. Of the black pens, all could be distinguished, apart from the Papermate samples; 16 and 18. Two of the red samples, Micron and Staedtler (9 and 11) could not be discriminated based on the APCI-MS identification alone.

The mass spectra obtained for the fresh samples of inks from pens removed from paper can be seen in Figures 3.2 – 3.5. A micropunch was used to cut disks out of the paper and methanol was used to extract the ink from the surface of the disks. It is clear to see in Figure 3.2 that the spectra for all of the blue samples are different and the peaks attributed to the dye components are the tallest. The spectra also show the relative abundance of these components in each pen. For the Woolworths pen, Basic Violet 1 and 3 are in abundance and Basic Blue 26 is less used, whereas for the Staedtler pen, the results are the opposite and Basic Blue 26 is the most abundant component. For the Bic pen, Basic Yellow 2 is the dominant peak followed by Basic Violet 3 and 1.

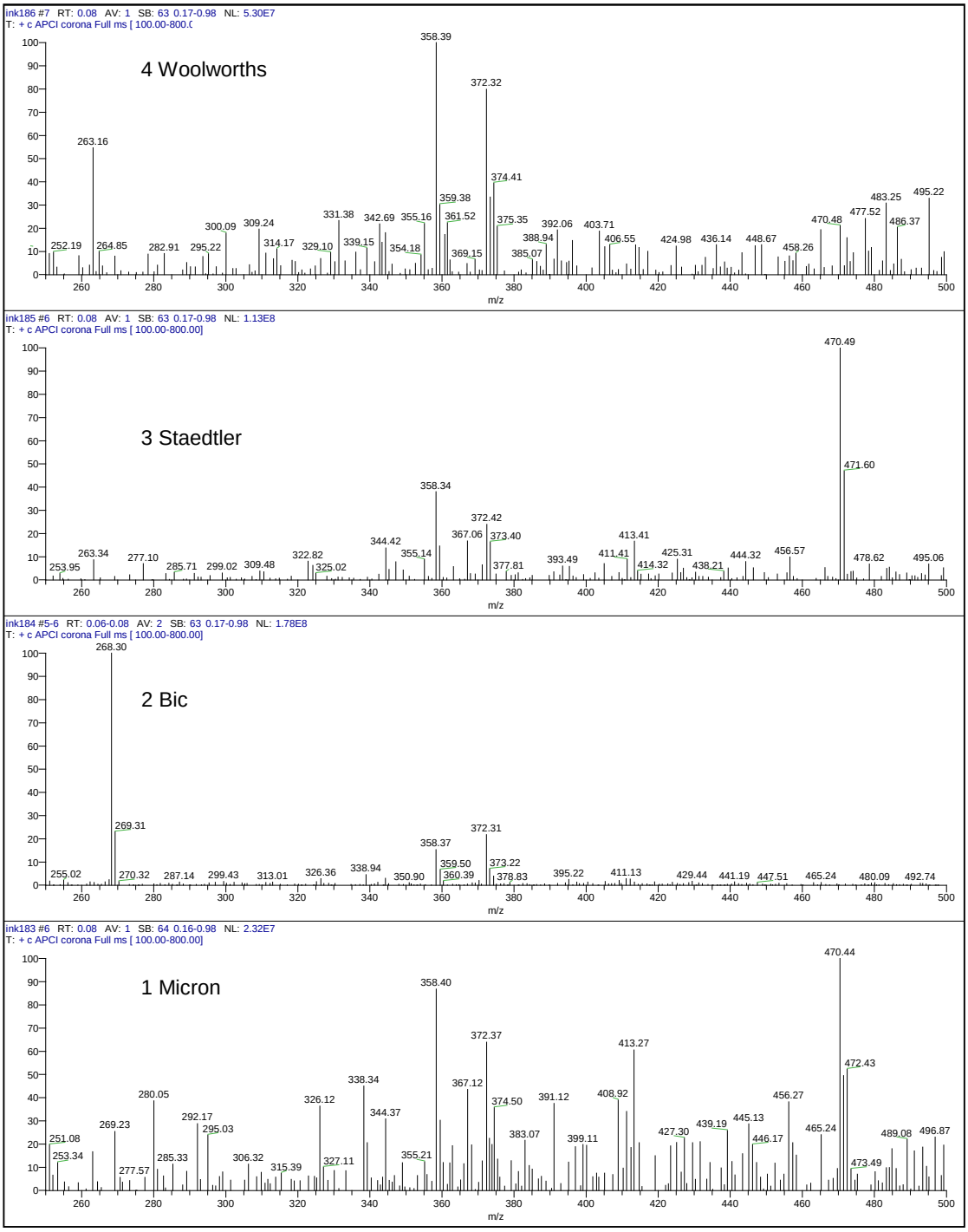


Figure 3.2 APCI-MS Spectra of Blue Ballpoint Pens

The main dye components in black ink are Basic Violet 3 and Basic Violet 1, but the intensities are different for each pen as can be seen in Figure 3.3.

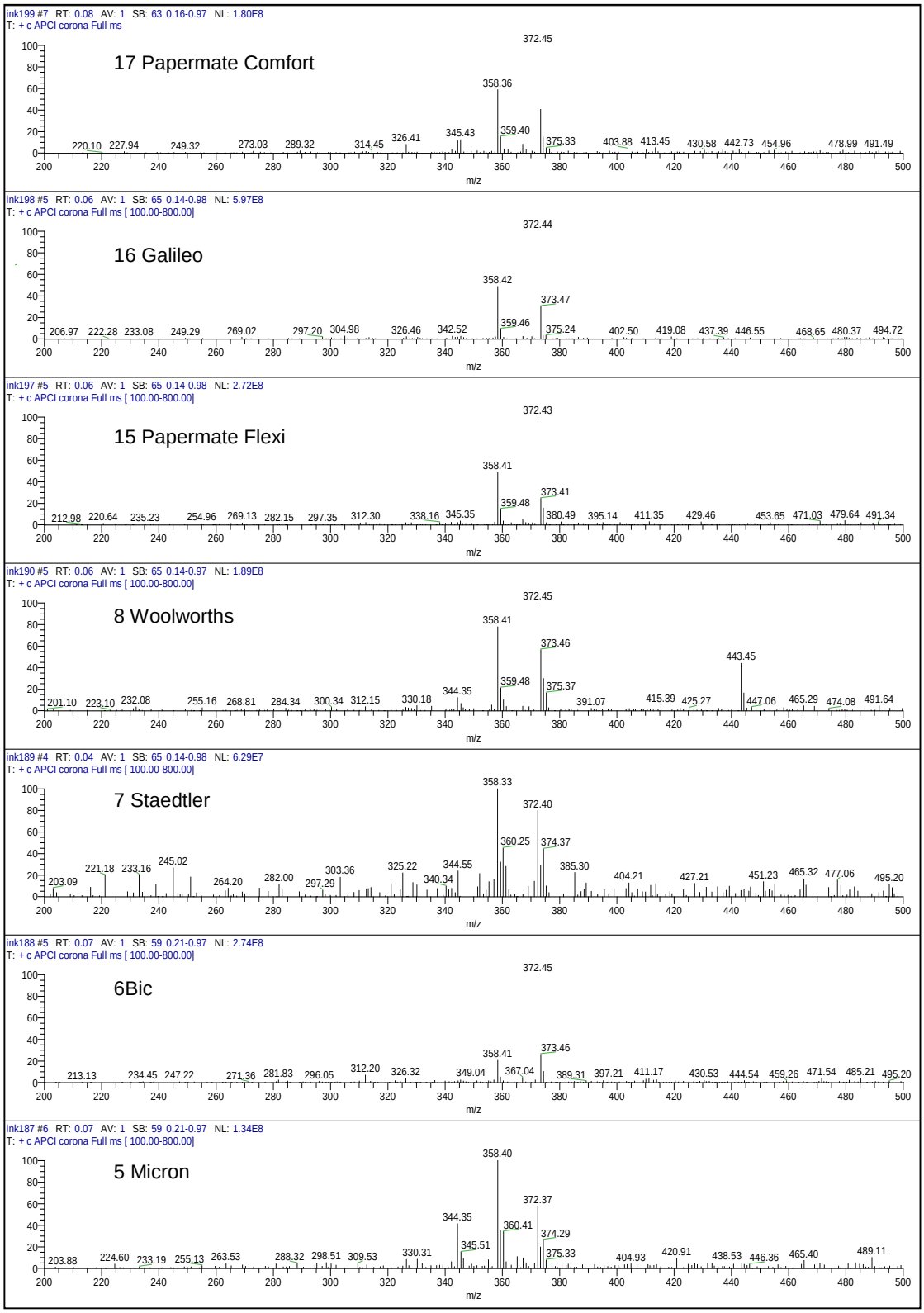


Figure 3.3 APCI-MS Spectra of Black Ballpoint Pens

In five of the seven black pens, Basic Violet 3 was more abundant than Basic Violet 1. The peak corresponding to Basic Red 1 at m/z 443 is clear to see in the Woolworths sample. Black ink may also contain carbon black to obtain the black colour.

Figure 3.4 shows the spectra for the red pens. The predominant peak is at m/z 443 which could be Basic Violet 10 or Basic Red 1. Analysis of the MS/MS spectra showed a combination of both dyes used in the pens. As with the blue pens, only the red Bic pen contains Basic Yellow 2 at m/z 268.

The composition of green inks is very different as can be seen in Figure 3.5. The identification of components present in these pens was not possible due to a number of unknown compounds. A peak at m/z 268 has been attributed to Basic Yellow 2 for the other coloured pens, but for green Bic, as there are also peaks present at m/z 212, 226, 240 and 254, these correspond to other aryl guanidines, so for green Bic m/z 268 is more likely to be dixylol guanidine than Basic Yellow 2. The purple ink contains m/z 268 also, but is identified as Basic Yellow 2. Basic Red 1 is responsible for the peak at m/z 443 in this pen.

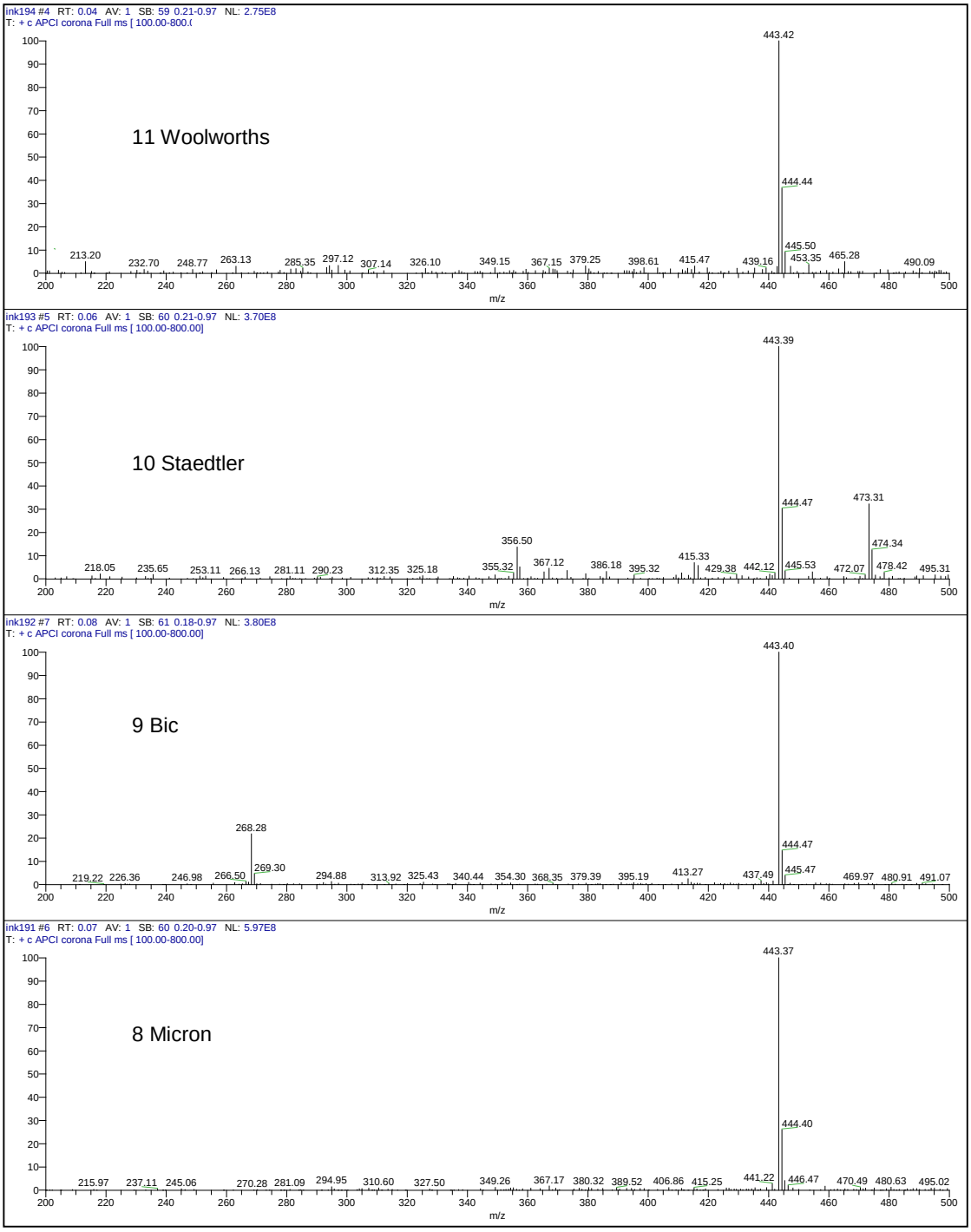


Figure 3.4 APCI-MS Spectra of Red Ballpoint Pens

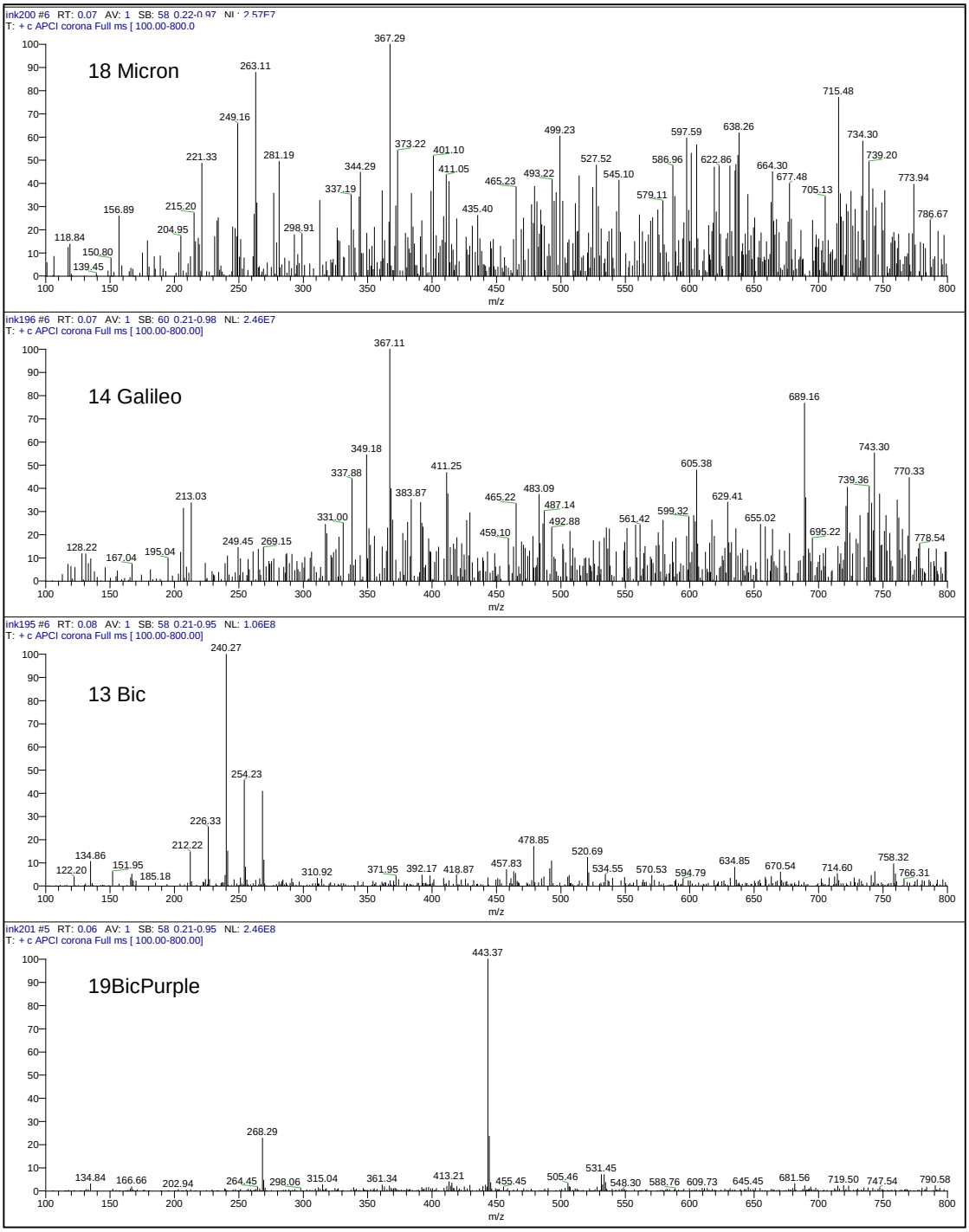


Figure 3.5 APCI-MS Spectra of Green and Purple Ballpoint Pens

3.3 Validation of Instrumentation

In order to determine the precision of the APCI-MS, the data for each dye standard was analysed and relative standard deviation (RSD) was obtained as shown in Table 3.2. RSD is used in analytical chemistry to assess the variation in data sets and the spread of data. A low number indicates little spread, a high number a lot of spread. As can be seen in Table 3.2 the RSD for all of the dye standards was between 10.13% and 17.42%. As these values were below 20%, they would be accepted according to recommendations set out for validation of analytical methodology for forensic samples by the United Nations.[147] These results indicate the levels of precision for the equipment used in this project, and allow the conclusions drawn from this research to be robust. The RSD values were not as low as would have been desired (less than 10%) which implies there was some variability in the results obtained for all of the samples. This could be due to numerous reasons but the main explanation being the varied use of the APCI-MS during the analysis of these samples, and the increased risk of contamination from other types of sample.

Table 3.2 Validation of APCI-MS technique using Dye Standards

	Dye Standard	M/z	Run			Total Ion Count/TIC	Average	Standard Deviation	RSD %
			A	B	C				
Data	BV3	372	11497972	13917090	14394540	39809602	13269867	1552965	11.70
	BV1	358	2276451	2992369	2554617	7823437	2607812	360911	13.84
	BB26	470	18693460	19824582	22706647	61224689	46086924	2069276	10.13
	BR1	443	6772306	9508881	7695164	23976350	7992117	1392245	17.42
	BV10	443	5987577	5169327	6843252	18000156	6000052	837031.9	13.95
Normalised Data	BV3	372/TIC	0.2888	0.3495	0.3615	1	0.333	0.03901	11.70
	BV1	358/TIC	0.2909	0.3824	0.3265	1	0.333	0.0461	13.84
	BB26	470/TIC	0.3053	0.3238	0.3708	1	0.333	0.0337	10.13
	BR1	443/TIC	0.2824	0.3965	0.3209	1	0.333	0.0580	17.42
	BV10	443/TIC	0.3326	0.2871	0.3801	1	0.333	0.0465	13.95

3.4 Discussion and Conclusions

Analysis of dye standards typically used in ballpoint pen ink was carried out using Atmospheric Pressure Chemical Ionisation Mass Spectrometry. The spectra showed the m/z value for the molecular ion of each dye clearly, but some of the dyes (Basic Violet 3 and 1) thought to be pure, actually contain a mixture of the dye homologues, for example Basic Violet 3 and Basic Violet 1 were both present in the spectra for the two, supposedly pure, dyes. Apparently, dyes can be sold as impure mixtures so long as the main component of the mixture is the stated dye.[26]

The information from the dye standards was then used to identify the dyes present in 19 ballpoint pen inks following APCI-MS of the ink on paper. Discrimination between all samples was not possible based on identification alone as multiple samples contained the same combination of dyes, but using quantitative data for the dyes, such as Relative Ion Count and Relative Proportions, discrimination was achieved as the dyes were present in different abundances. Intensity and relative abundance information obtained from the mass spectra was used to determine relative proportions of each dye. For the blue inks, both Basic Violet 3 and Basic Violet 1 were present in all pens, with Basic Violet 1 the most abundant in all samples, except for the Bic pen where Basic Violet 3 was in abundance. When another dye was also present and was the base peak, for example, Basic Blue 26, Basic Violet 1 was still present in higher quantities than Basic Violet 3. With regards to the black inks, it varied between both violet dyes as to which was the most abundant, however the results were consistent for blue and black of the same brands. The black Papermate samples could not be discriminated by qualitative or quantitative means suggesting the same or very similar ink formulation is used for multiple pen products of the same brand and possibly other brands. With the red and green inks, discrimination was possible just using qualitative information.

The results have shown that manufacturers use different combinations of dyes to achieve their colour of choice, but also that numerous dyes can be present in a dye mixture, as well as the other typical ingredients used such as solvents and

resins. It is possible that manufacturers use similar recipes for different products, such as the blue and black inks and get the difference in colour by addition of another dye. This would explain why the composition of the inks with regards to Basic Violet 3 and Basic Violet 1 was so similar between colours of the same brand. This is not certain, however, as only one sample of each colour from each manufacturer was analysed in this work.

Analysis of dye standards and fresh ballpoint ink samples has enabled identification of the dyes present in the inks and indicate a novel method for distinguishing between various manufacturer's inks. Mass spectra can be difficult to analyse especially if there are multiple peaks present. It can be extremely difficult to make identifications of the peaks as there are numerous possibilities as to the identification for any m/z value. Identification of the individual dyes can be made using ms/ms on each dye molecular ion peak. However it is not really feasible for routine easy identification as there are often several isomers with the same mass and the resultant ms/ms of the peak would be a mixture of the isomer fragmentation products. Attempts at using liquid chromatography/mass spectrometry to solve this problem proved unreliable and for this research the focus was on the degradation products, therefore this line of enquiry, even though it showed promise for ink discrimination, was not pursued further.

The purpose of this research was to monitor the aging process of the inks using APCI-MS and identify the degradation products of the faded samples. Degradation pathways of many individual dyes have been reported in the literature,[137,138,148,149] but as mentioned previously, inks contain multiple dyes which may have a synergistic or antagonistic influence on each other and as the published pathways focus on individual dyes these observations are of limited use. Furthermore, degradation can occur due to numerous reasons such as light, pH, oxidising or reducing chemicals and heat, all of which might affect the pathway in different ways. Controlled experiments are required to monitor the degradation and the effects of different variables.

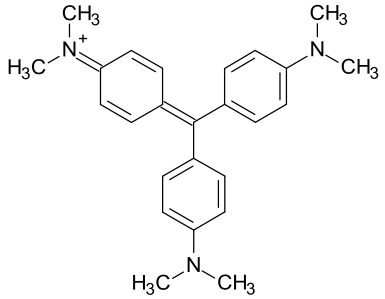
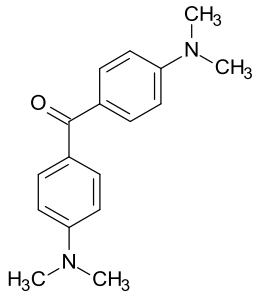
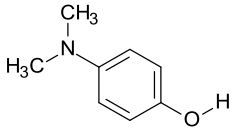
Chapter 4 Analysis of Blue Inks

This Chapter will look at the degradation of dye standards, mixtures and inks of blue pens when subjected to light, peroxide and bleach.

4.1 Degradation Products

As identified in Chapter 3, the blue pens predominantly consist of Basic Violet 3, Basic Violet 1 and Basic Blue 26. Typical degradation products for Basic Violet 3 and Basic Violet 1 are shown in Table 4.1 and Basic Blue 26 in Table 4.2. As structurally both Basic Violet dyes are very similar, their degradation will follow the same pattern. The products in the Tables are possible fragments that correspond to m/z as derived from APCI data and relevant literature and are likely to be seen in the APCI spectra for the samples.

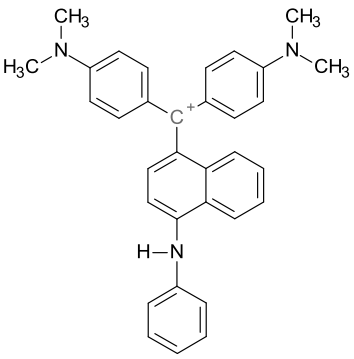
Table 4.1 Possible Degradation Products for Basic Violet 3 and Basic Violet 1

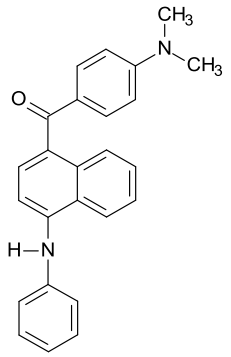
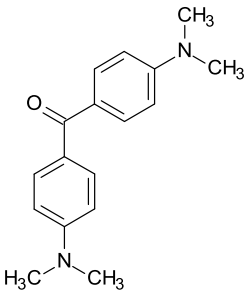
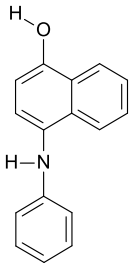
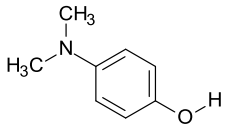
Structure	m/z	Compound
	372	$C^+ (Me)_6$ (BV3)
	358	$C^+ (Me)_5H$ (BV1)
	344	$C^+ (Me)_4H_2$
	330	$C^+ (Me)_3 H_3$
	316	$C^+ (Me)_2 H_4$
	302	$C^+ (Me)_1 H_5$
	288	$C^+ H_6$
	268	N,N-Dimethylaminobenzophenone (DDBP$(Me)_4$)
	254	DDBP $(Me)_3$
	240	DDBP $(Me)_2$
	226	DDBP $(Me)_1$
	212	DDBP H_4
	137	N,N-Dimethylaminophenol (DAP)
	123	DAP $(Me)_1$
	109	DAP H_2

The initial reactions that take place are due to *N*-demethylation – the loss of methyl (-CH_3) groups from the nitrogen group. As Basic Violet 3 has six methyl groups attached to the aromatic amine substructures of the cation (C^+), there could be a successive loss of all six of these groups, with multiple isomers occurring for the di-, tri- and tetra-demethylated molecules. Basic Violet 1 has five methyl groups, so can only lose those five. There is a change of 14 atomic mass units as photooxidation converts the N-methyl groups to methanal or methanoic acid, effectively replacing the methyl groups by hydrogen atoms (-H). This is known as oxidative demethylation.[35,38,66] Another possible pathway for degradation is via cleavage of the central carbon-aryl bond which leaves a benzophenone product.[35,64] These too may have various numbers of methyl groups attached, so successive loss of these groups can also be seen. The complementary part of the structure forms a phenol as shown in Table 4.1.

Basic Blue 26 as another triarylmethane dye has a similar structure to Basic Violet 3, as shown in Table 4.2. *N*-Demethylation occurs with the loss of four -CH_3 groups and some of the same benzophenone and phenolic products as formed by Basic Violet 3 can be formed by Basic Blue 26. In addition, as the two dye structures are not completely the same, Basic Blue 26 can produce added degradation products in the form of ketones and phenols as outlined in Table 4.2.

Table 4.2 Possible Degradation Products for Basic Blue 26

Structure	m/z	Compound
	470	C^+ $(Me)_4$ BB26
	456	C^+ $(Me)_3$
	442	C^+ $(Me)_2$
	428	C^+ $(Me)_1$
	414	C^+ H_4

	366	(4-anilino-1-naphthyl)-(4dimethylaminophenyl)methanone (ANDAPM) $(Me)_2$
	352	ANDAPM $(Me)_1(H)_1$
	338	ANDAPM $(H)_2$
	268	N,N-Dimethylaminobenzophenone DDBP $(Me)_4$
	254	DDBP $(Me)_3$
	240	DDBP $(Me)_2$
	226	DDBP $(Me)_1$
	212	DDBP H_4
	235	4-anilino-1-naphthol
	137	N,N-Dimethylaminophenol (DAP)
	123	DAP $(Me)_1$
	109	DAP H_2

The mass signals (m/z) of the spectra from all samples were compared to perform a preliminary classification based on qualitative information, e.g. presence/absence of peaks. The relative signal intensities of the results were then used for further analysis to determine the approximate relative proportions of each component (RP). This is based on the relative peak area (RPA) used in earlier studies on dye degradation.[70,150] Grim also used relative intensities to monitor degradation by UV irradiation.[26] Average molecular weight of the dyes over time was also used in that study but it would not be as useful here as specific degradation products are being investigated to understand the ageing process, and average molecular weight does not allow as accurate an identification.

For the purposes of this study, RP can be defined as

$$RP_{\mathrm{i}} = \frac{I_{\mathrm{i}}}{I_{\mathrm{tot}}} \times 100$$

Where I_i is the relative intensity of the peak signal at m/z = *i* and I_{tot} is the total intensities of all the signals (molecular ion and degradation products) of the dye. With this definition it is possible to define aging curves as a plot of RP as a function of time. For example,

$$RP_{372} = I_{372} \,/\, (A_{372} + A_{358} + A_{344} + A_{330} + A_{316} + A_{302} + A_{288})$$

RP provides important information which emphasises the relative stability of the dye compounds.

The extent of the fading can be related approximately to the total ion count of all of the components seen which will be associated with the concentration of the sample. The concentration will change over time and when samples have been subjected to artificial aging. This semi-quantitative method will be used to estimate the degradation of the dyes with time and the approximate proportions of the potentially useful benzophenone and phenolic products.

4.2 Photodegradation

The dye standards were faded photochemically in solution and ink was faded on paper by the methods described in Chapter 2. The photodegradation products of both the pure dyes and the ink mixtures were analysed by APCI-MS over six days for the standards and 10 days for the mixtures to monitor the aging and fading processes and to determine whether there were any synergistic effects of one dye on the fading of another, e.g. by one dye producing excessive amounts of singlet oxygen which can then attack and increase the fading rate of other dye molecules.

4.2.1 Photofading of Dye Standards in Solution

Each dye standard was faded using an artificial daylight source as described in Chapter 2 to replicate the degradation process of natural photofading. Figure 4.1 shows the aging curves for demethylation for Basic Violet 3 and Basic Violet 1 along with the benzophenone degradation products.

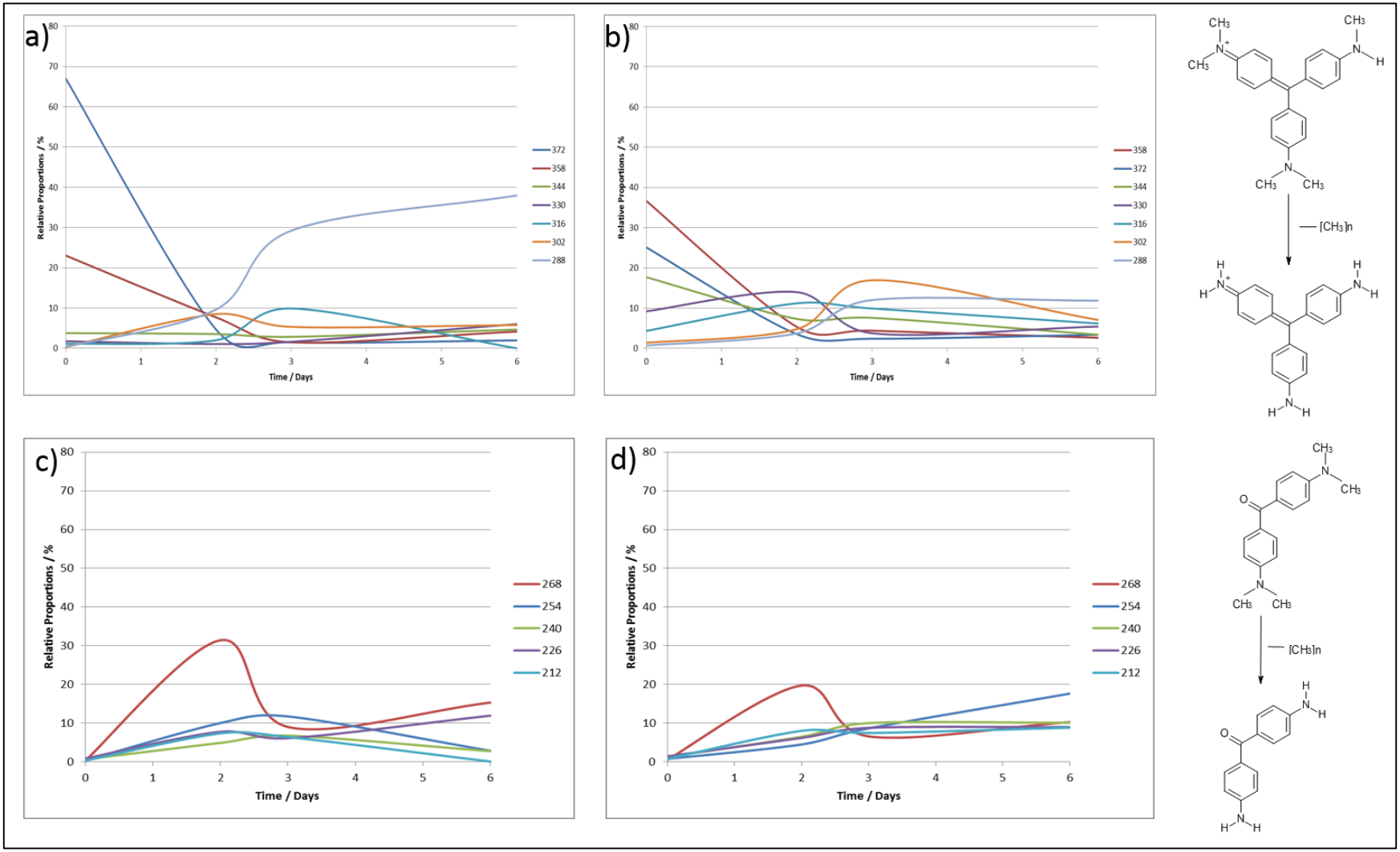


Figure 4.1 Aging Curves for dye standards a: Basic Violet 3 b: Basic Violet 1, c: Benzophenones for BV3 d: Benzophenones for BV1 during Photofading

Graphs a) and b) in Figure 4.1 show that the relative proportions of the main dye components, Basic Violet 3 (6 Me) and Basic Violet 1 (5 Me), decreased significantly over time. This was supported by the relative ion count data which shows that the degree of degradation was quick for both dyes. At T = 0 both dyes were 100% but at T = 6 days Basic Violet 3, m/z 372 was 2.5% and Basic Violet 1 m/z 358 was 7.5%. This means that significant degradation of both dyes took place in just six days for their values to reduce by these amounts, and the degree of degradation was almost the same for both dyes. The relative proportion of the lower homologues 288 (0 Me) showed a significant increase compared to the other products, which would not have been expected until the higher homologues had degraded. This was probably due to a combination of two factors: (i) the hypsochromic shift (to higher energy) of the absorption as the dyes demethylate progressively and (ii) the proportion of the light from the artificial daylight source decreases as it goes to higher energy, resulting in a lower rate of degradation. As the RP values were small for all homologues and of similar value, this indicates that apart from m/z 288, no homologue contributed more to the composition than any other. This was completely different for Basic Violet 1 as all homologues apart from m/z 344 increased immediately and there was a clear succession in their production and peak RP values. The demethylation products m/z 330 (3 Me) and 316 (2 Me) increased initially which would be due to other higher homologues breaking down and producing them, but then they themselves break down explaining the subsequent decrease in their proportions. Relative ion count data for the homologues showed that the values were much higher for Basic Violet 1 than 3. M/z 330, 316 and 288 reached values of 70%, 56%, 37% respectively during the time, but for Basic Violet 3 the same components never exceeded 28%. This would indicate that the degradation of Basic Violet 1 was faster than Basic Violet 3 as the degradation products constituted much higher proportions. These two dyes had not completely faded in six days, but they were colourless by eight days of exposure to light.

Graphs c) and d) in Figure 4.1 show the other degradation products that were produced by cleavage of the central phenyl bond. The most abundant component at two days for both dyes was m/z 268 (4 Me) with a RP of 32% and 20% respectively indicating that it was produced quickly once the parent dye was subjected to light. This would be the first benzophenone expected as it has 4 CH_3

groups still attached. The higher value for m/z 260 in Basic Violet 3 could correspond to the significant drop in m/z 372 over the first 2 days seen in graph a), which was not as dramatic for Basic violet 1 in graph b). The other products also increased in proportion but increased for the six days for Basic Violet 1. M/z 254, 240 and 212 for Basic Violet 3 decreased in RP after three days. All products were still being produced in slightly higher proportions for Basic Violet 1 rather than 3. This can be seen clearly in Figure 4.2. This is corroborated by the relative ion count data, as all values were higher for Basic Violet 1 supporting that degradation was faster for this dye. At T = 6 days, values for Basic Violet 1 ranged between 25% – 50%, but were only 1% – 20% for Basic Violet 3.

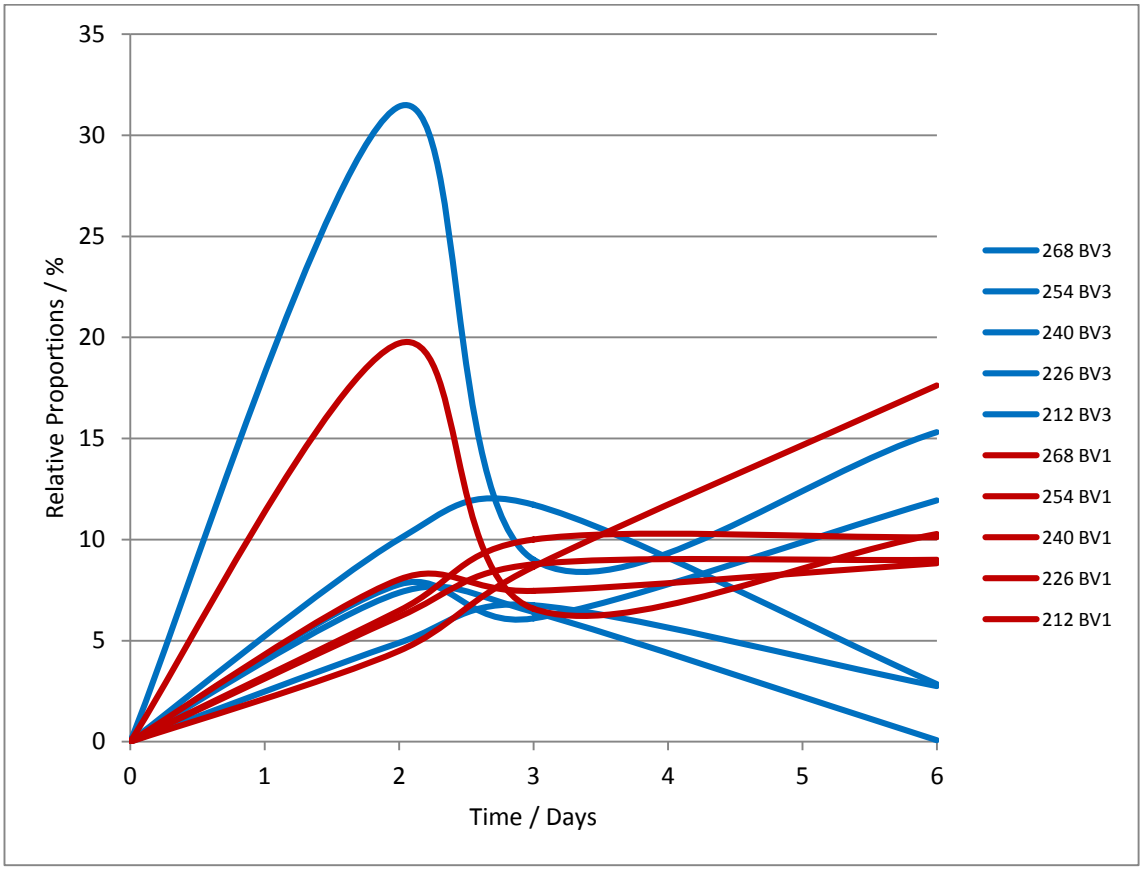


Figure 4.2 Aging Curves for Benzophenone Products from Basic Violet 3 and Basic Violet 1 during Photofading

Phenol products were not detected in high enough quantities to confirm their presence for either of the dyes.

Figure 4.3 shows the aging curves for Basic Blue 26. There appeared to be a rapid reduction in RP value for m/z 470, but this did not produce significant degradation products as seen with Basic Violet dyes.

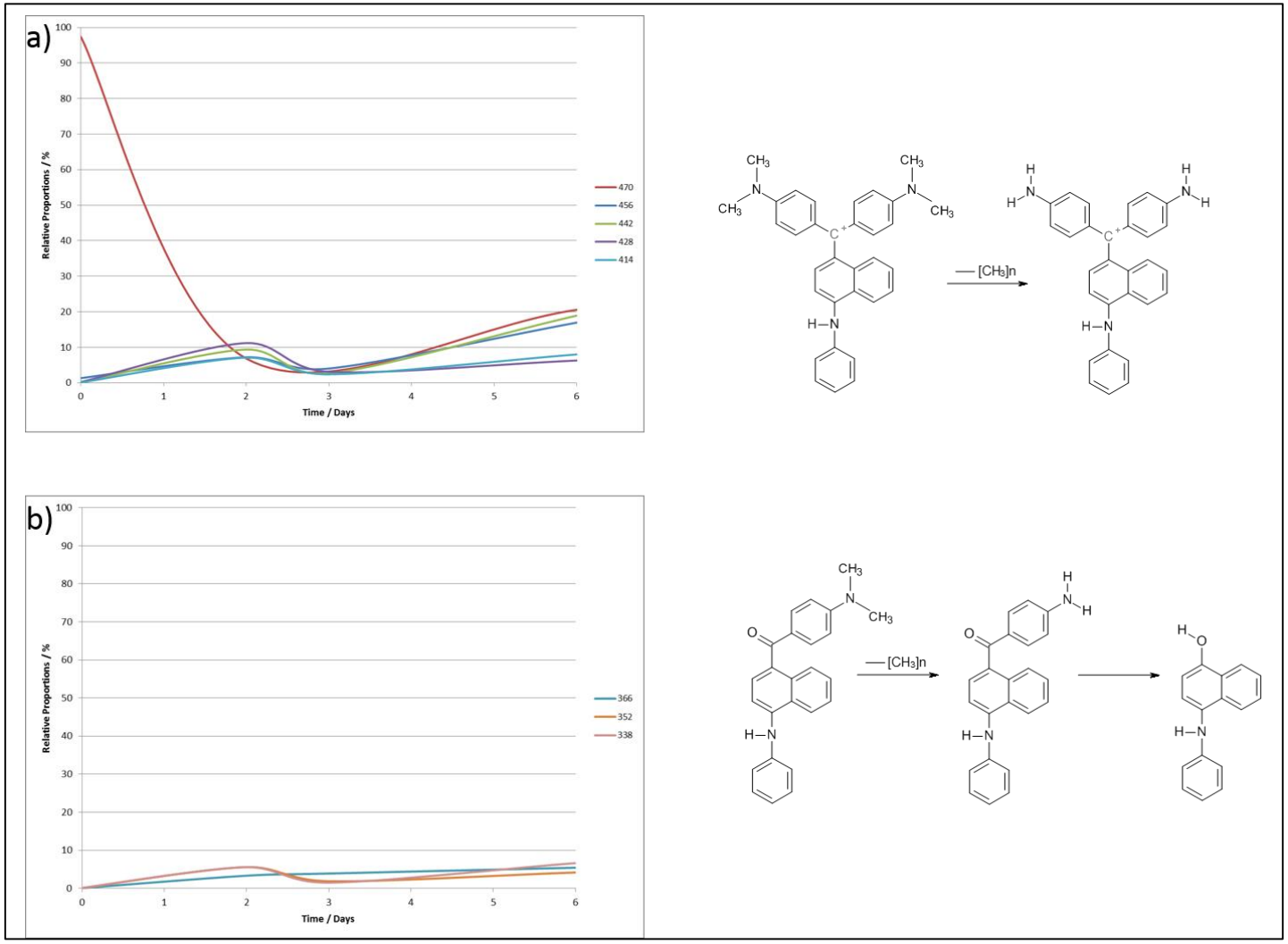


Figure 4.3 Aging Curves for dye standard a: Basic Blue 26, b: Benzophenone for BB26 during photofading

Table 4.3 shows the relative proportions of the component types when fresh and after six days.

Table 4.3 Table to show the Relative Proportions of Products based on Ion Count Data after Photofading

	Basic Violet 3		Basic Violet 1		Basic Blue 26	
	T=0	**T=6**	**T=0**	**T=6**	**T=0**	**T=6**
Dye /%	97	60	95	40	99	70
Benzophenones /%	3	34	5	55	0	30
Phenols /%	0	6	0	5	0	0

The data shows a reduction in all of the dye components, and a greater reduction for Basic Violet 1 than Basic Violet 3. Basic Violet 1 reduced from 95% to 40%, and Basic Violet 3 was 97% to 60%. The benzophenone products increased for all dyes, with once again a greater increase for Basic Violet 1, however, the phenol increase was almost the same for both violet dyes at 5% and 6%. This information would suggest that benzophenone and phenol products were present.

4.2.2 Photofading of Dye Mixtures in Solution

By using dye combinations identified from the ink compositions of the blue ballpoint pen samples shown in Table 3.1, 3 blue dye standard combinations were produced as displayed in Table 4.4 for analysis to replicate ink degradation by light. APCI-MS analysis was carried out over 10 days and aging curves produced of the results.

Table 4.4 Dye Standard Mixtures

Dye Standard Mixture	Dye Standard Proportions	Pen Colour	Pen number
1	BV1 20 : BV3 15	Blue	2
2	BB26 100 : BV3 50	Blue	1
3	BV1 100 : BV3 70 : BB26 20	Blue	4

It can be seen in Figures 4.4 that for all 3 mixtures m/z 372 and 358 decreased in relative proportion over time, and the other components; m/z 344, 330, 316, 302 and 288 increased in their RP values which was consistent with degradation and due to photofading. Mixture 1 contained 2 dyes of almost identical composition; (Basic Violet 1 and Basic Violet 3), and they follow the same degradation pathway. This means there were two dyes producing the same degradation products. Mixture 2 also contained 2 dyes but their structures were not the same; (Basic Violet 3 and Basic Blue 26) and produce different degradation products. This could explain why the RP values for Basic Violet components in mixture 1 were generally higher than the RP values for the same components, as mixture 2 only contains 1 Basic Violet dye.

Mixture 3 contained 3 dyes; Basic Violet 1, Basic Violet 3 and Basic Blue 26, but Basic Violet 1 and 3 were in almost the same ratio as in mixture 1. However their aging curves were not exactly the same. The RP values were higher than mixture 2 probably due to 2 dyes contributing to the same degradation products, but the values were also higher than mixture 1. This could be due to the involvement of Basic Blue 26 which was present in mixtures 2 and 3.

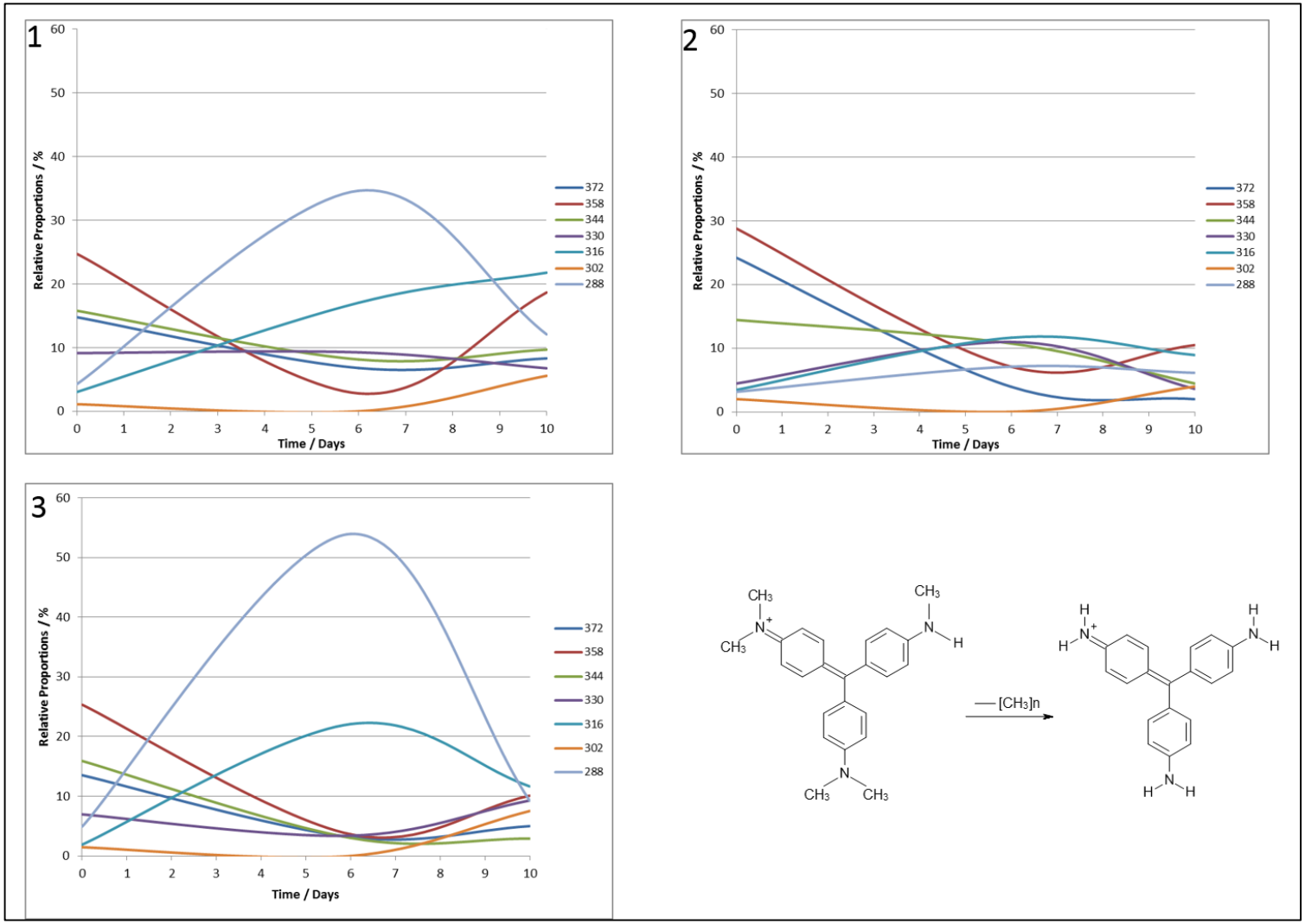


Figure 4.4 Aging Curves for Basic Violet Dyes in Dye Mixtures 1, 2 and 3 during Photofading

Mixture 2 contained more Basic Blue 26 than mixture 3, but also in mixture 2 it was the principal component, in mixture 3 it was smallest component. This seems to be contributing to the degradation of the Basic Violet components. When there was more Basic Blue 26, the degradation products were produced earlier than when there was less Basic Blue 26. This was the first observation of a synergistic fading effect of one dye upon another. The relative ion count data showed that m/z 372 for both mixtures actually degraded to the same proportion to be 3% at T = 10 days. The values for the homologues were much higher for mixture 2 for the earlier ones (m/z 344, 330), but higher in mixture 3 for the later ones (m/z 316 and 288). This is unusual as you would not expect the later homologues until the higher homologues have degraded. This was probably due to a combination of two factors: (i) the hypsochromic shift (to higher energy) of the absorption as the dyes demethylate progressively and (ii) the proportion of the light from the artificial daylight source decreases as it goes to higher energy, resulting in a lower rate of degradation.

For mixtures 2 and 3, Basic Blue 26 appeared to behave in the same way as shown in Figure 4.5. M/z 470 decreased steadily in proportion over time and the homologues were gradually increasing indicating that degradation was occurring. Relative ion count data showed that m/z 470 decreased from 100% at T = 0 to 3% at T = 10 days for both mixtures. This would indicate that the amount of degradation for Basic Blue 26 was the same for both mixtures regardless of ratio of dyes.

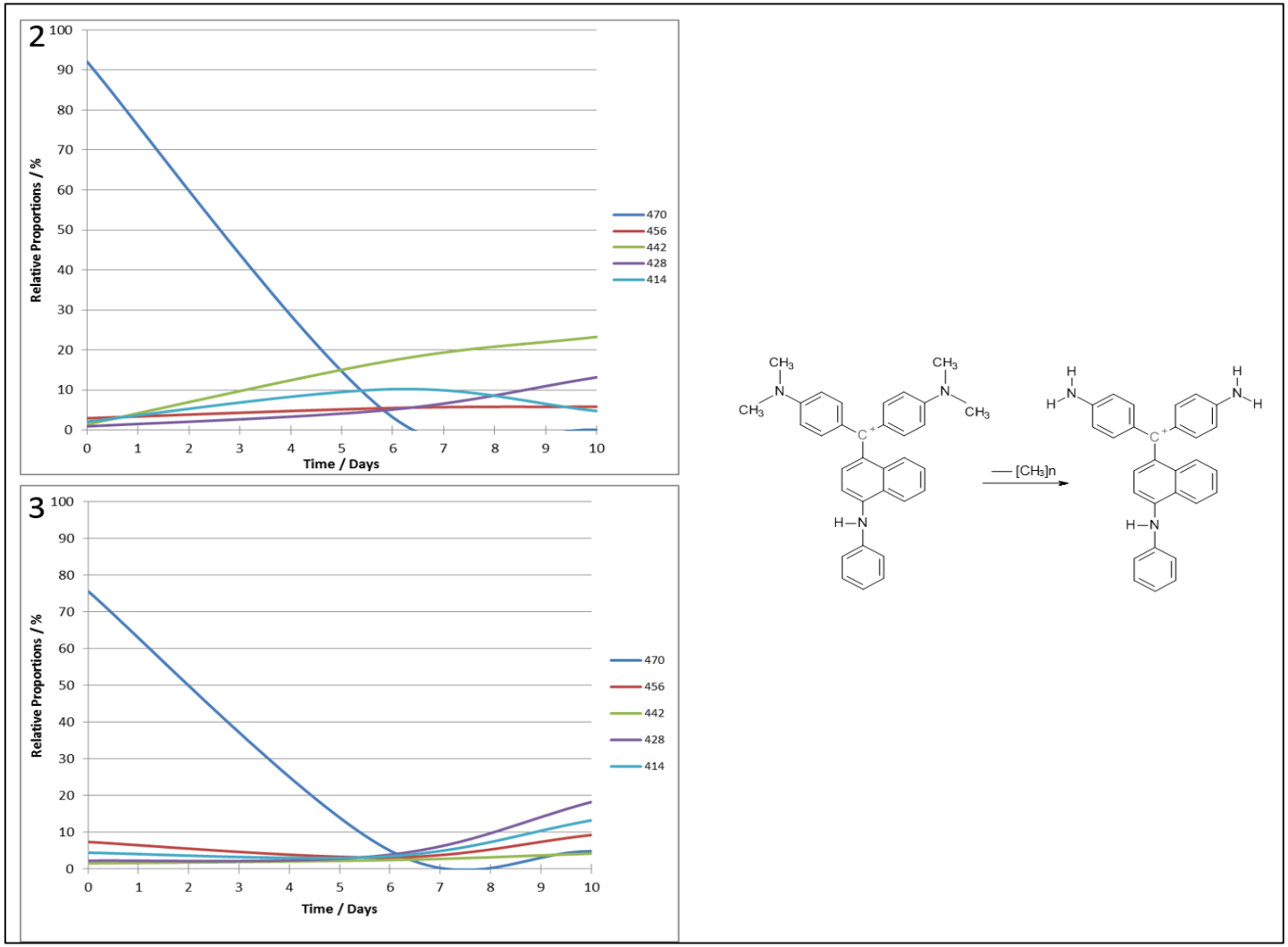


Figure 4.5 Aging Curves for Basic Blue 26 in Dye Mixtures 2 and 3 during Photofading

Table 4.5 displays relative ion count data for m/z 372 and 358 for mixtures 1, 2 and 3. It is clear to see that both dyes reduced in proportion over the ten days and mixture 1 by the greatest amounts.

Table 4.5 Table to show Relative Proportions from Ion Count Data for m/z 372 and 358 for Mixtures 1, 2 and 3 after Photofading

Mixture	M/z 372		M/z 358	
	T = 0	T = 10	T = 0	T = 10
1	44	7	74	15
2	42	15	50	15
3	30	9	48	14

As mentioned, benzophenone products can be produced following degradation of dye molecules. Figure 4.6 displays the aging curves for these products in dye mixtures 1, 2 and 3 after photofading.

Unlike with the parent dye components, in mixture 1 the RP values for the benzophenone components were low. This could be due to the incomplete degradation of the parent dyes and therefore no production of benzophenone products.

In mixture 2 the benzophenones were produced almost immediately and their relative proportions were much higher than in mixtures 1 and 2. This strongly suggests that degradation was faster for mixture 2 (which contained more Basic Blue 26) than mixtures 1 and 3 which is consistent with the parent dye data. Relative ion count data supports this as values for mixture 2 were at their highest around five days, but the highest values for mixture 3 were at 10 days.

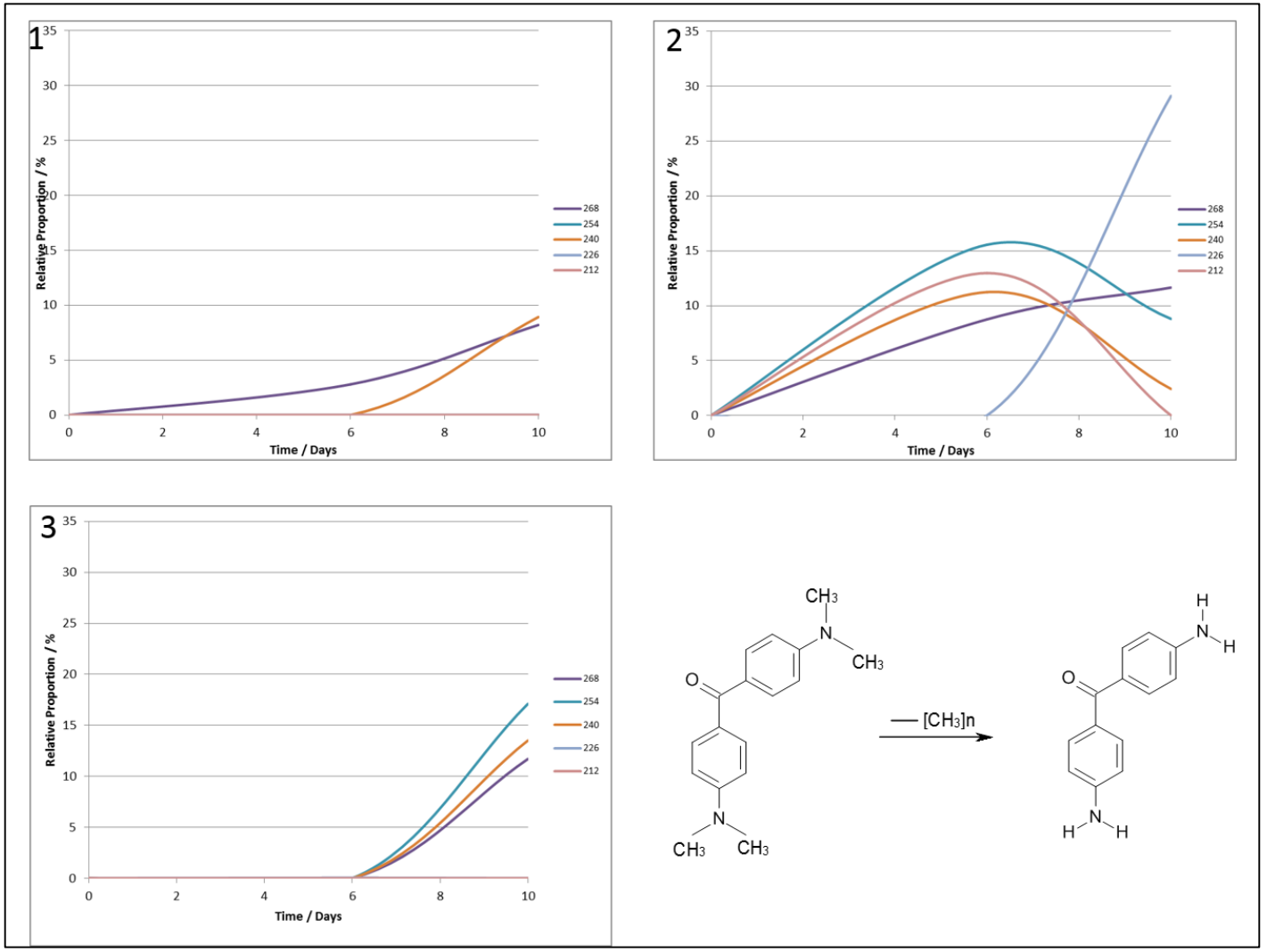


Figure 4.6 Aging Curves for Benzophenones Products from Basic Violet 3 in Dye Mixtures 1, 2, and 3 during Photofading

For benzophenones associated with Basic Blue 26, they are shown in Figure 4.7 Their behaviour appears similar for both mixtures 2 and 3, but for m/z 235 the RP is much higher in mixture 3 which contains less Basic Blue 26. The reason for this is unexplained at the current time as the degradation of Basic Blue 26 parent components appeared similar for both mixtures.

From the results of the photofading of the dye mixtures, it appears that Basic Blue 26 when present in larger quantities than other dyes can speed up the degradation process of the Basic Violet dyes. It has been reported in the literature that dyes produce singlet oxygen when excited by light, [64,66,67] which can then catalyse the degradation of other dyes such as Basic Violet 3 and Basic Violet 1.[151] It is therefore likely that Basic Blue 26 was producing single oxygen during the fading process which then attacks the more susceptible Basic Violet dye molecules.

No phenolic compounds were detected in high enough quantities to confirm their existence for any of the mixtures.

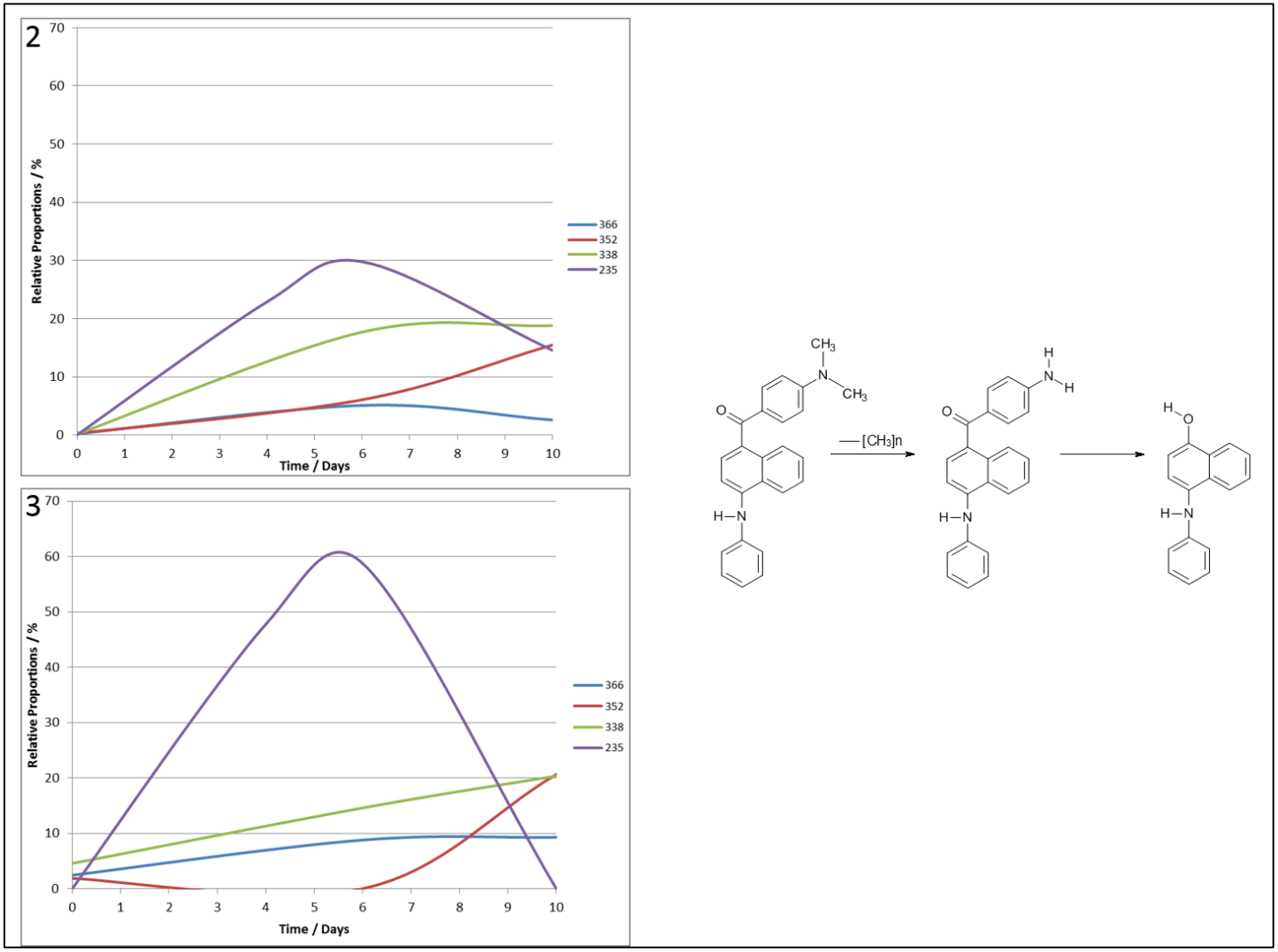


Figure 4.7 Aging Curves for Benzophenone Products for Basic Blue 26 in Dye Mixtures 2 and 3 during Photofading

Table 4.6 shows the relative proportions of the component types in the dye mixtures when fresh and after 10 days based on the ion count data. The reduction in dye components over time can be seen, but the amount of change was different for each mixture with mixture 2 having the greatest reduction in dyes and increase in benzophenones.

Table 4.6 Table to show the Relative Proportions of Products based on Ion Count Data in Dye Mixtures after Photofading

	Dyes /%		Benzophenones /%		Phenols /%	
Mixture	**T = 0**	**T = 10**	**T = 0**	**T = 10**	**T = 0**	**T = 10**
1	82	80	18	20	0	0
2	97	48	1	52	0	0
3	80	55	20	45	0	0

4.2.3 Photofading of Inks on Paper

The ink samples were placed indoors against a window facing northeast for natural sunlight and they remained there until the ink had faded. For 106 days from March to July, the light that the samples were subjected to was recorded using an *EDDI Peak* meter. Figure 4.8 shows the results obtained for both the temperature and light for this period of time. The temperature in the room throughout this period stayed between 17°C and 29°C, the temperature could not be controlled. For the light, there was a considerable amount of fluctuation during this time period with light values ranging from 87 to 1425 Lux, however the average Lux during this time is indicated on Figure 4.8 as 354 Lux. As would be expected, the temperature and light would also vary during the course of a day.

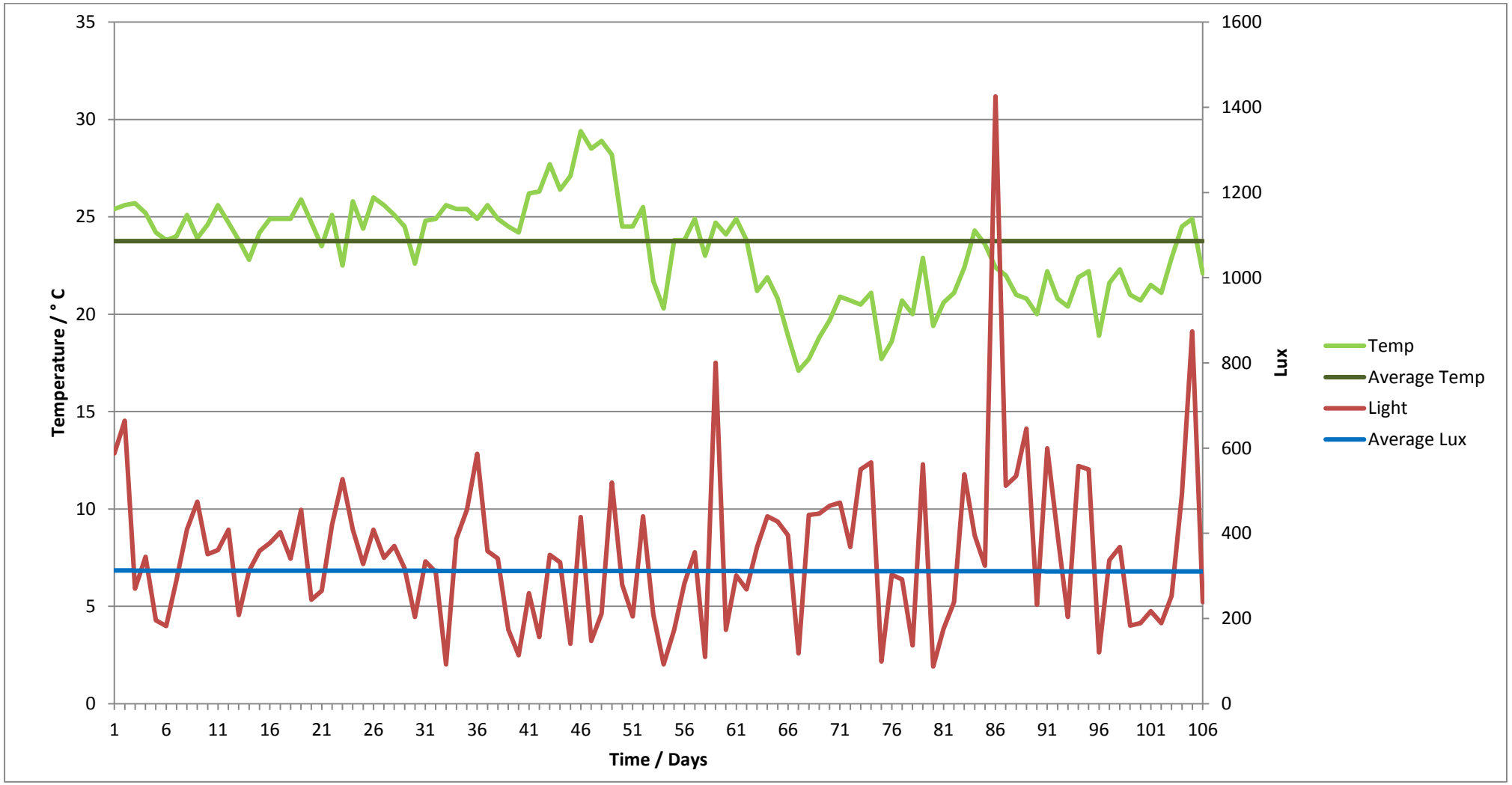


Figure 4.8 EDDI Peak data showing Temperature and Light Measurements from March to July 2008

The ink samples all degraded at different rates. Figure 4.9 shows what the blue inks looked like to the unaided eye after 15 months using a Video Spectral Comparator 5000.

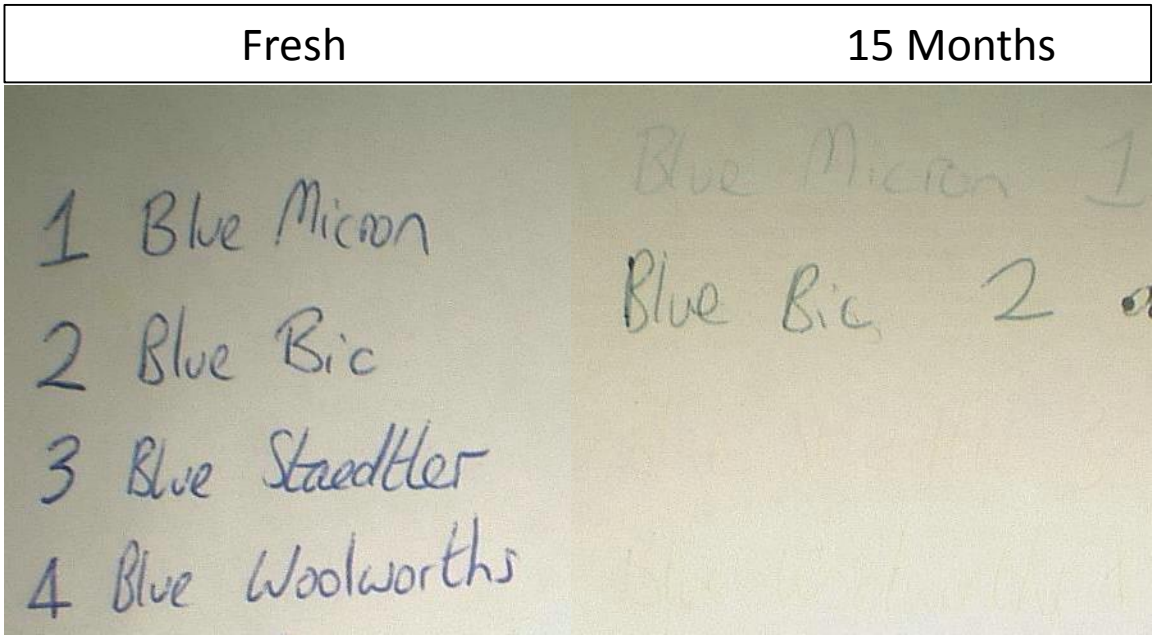


Figure 4.9 Image of Fresh Blue Ink and Ink exposed to daylight for 15 months.

Two of the blue pen samples Staedtler and Woolworths were no longer visible; their colour had in fact completely disappeared by 11 months. For the Staedtler, the blue faded to black before becoming colourless, whereas the Woolworths faded to brown. The VSC was used to look at the absorbance, reflectance and fluorescence of the samples before and after photodegradation. No changes were identified for any inks for IR absorbance or reflectance. Figure 4.10 shows the results from fluorescence analysis of blue ink samples after 23 months.

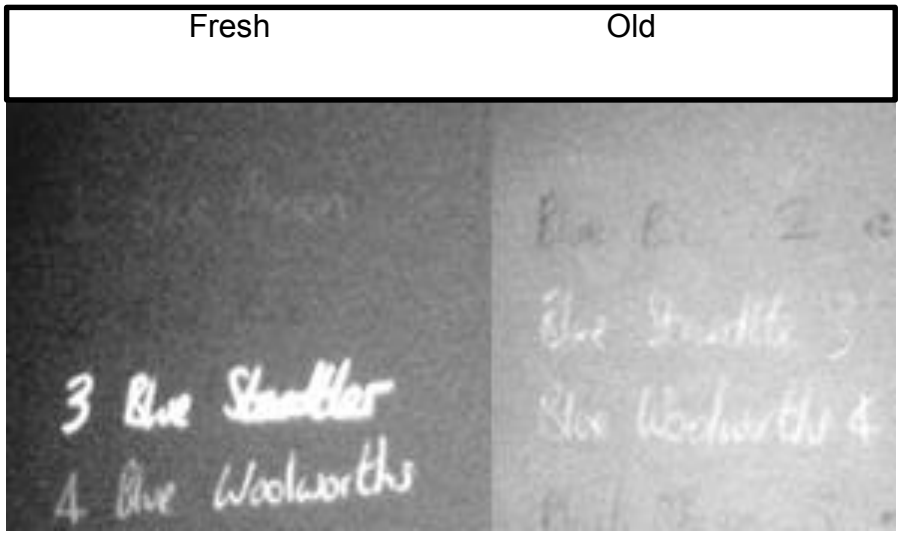


Figure 4.10 VSC Fluorescence images for Blue Pen Samples (Fresh and Old)

The samples that fluoresced when fresh were 3 and 4. What can be seen in Figure 4.10 is that when the samples start to degrade and fade the fluorescence characteristics can change. The intensity of the fluorescence was less for both samples after photofading, but no additional fluorescence was present. Under visible light, samples 3 and 4 no longer had any colour and were virtually unreadable. Using the VSC the fluorescence of these samples allowed the writing to be visualised which is a novel approach for ink analysis.

There was not one common dye that was present in all of the samples that fluoresced, but in samples 3 and 4 Basic Blue 26 was present. The structure of Basic Blue 26 as seen in Table 1.2 contains a naphthalene system. If there are two conjugated benzene rings like in naphthalene this means there is an extended π system and the molecule will fluoresce. Further investigation is needed in this area to determine what was causing the fluorescence to develop in some samples after photofading. This could be extremely valuable information as it could be used to re-image any ink that contains that specific fluorophore without chemical intervention.

If faded writing has been written over with a different ink, this technique may be able to re-visualise the original ink. This method is used in document examination to detect alterations and additions, but it has not been reported for the use of faded inks.

Ink samples were analysed using APCI-MS over 23 months and Figure 4.11 displays the aging curves for the dye Basic Blue 26 which was one of the dyes present in pens 1, 3 and 4 – Micron, Staedtler and Woolworths respectively. A fluctuating pattern can be clearly seen for the components indicating degradation of higher homologues into lower homologues. M/z 470 decreased in proportion very quickly for all pens and the lower homologues, particularly m/z 414 (0 Me) became the most abundant product over time. All Basic Blue 26 components degrade into m/z 414 which would explain the high RP values for this particular component.

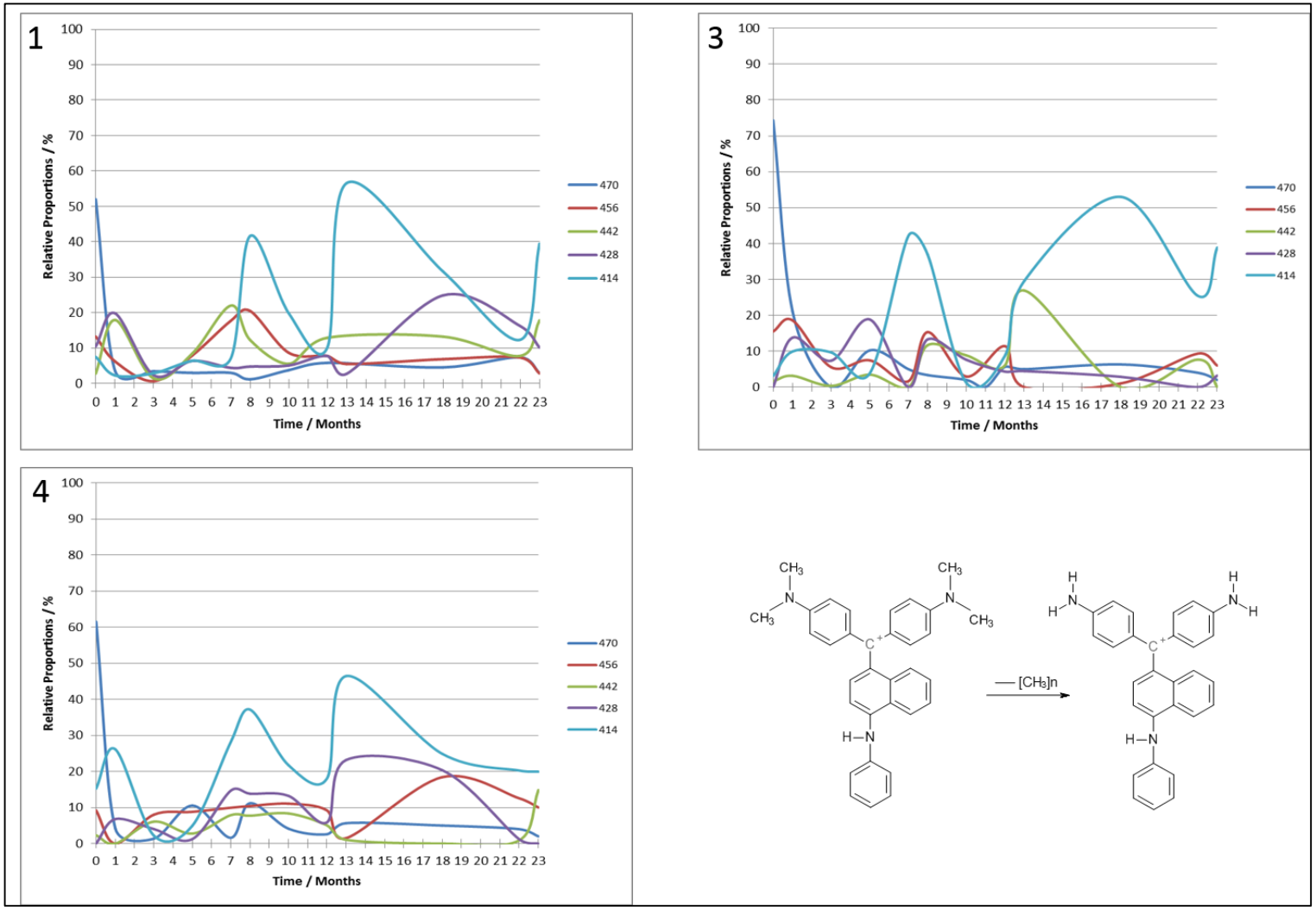


Figure 4.11 Aging Curves for Basic Blue 26 in Blue Pens 1: Micron, 3: Staedtler, 4: Woolworths after 23 months exposure to sunlight

It is interesting to see also that the products were continually produced and degraded, and that it was not always the successive degradation from m/z 470 to m/z 414. The aging curves were very similar for all three pens indicating consistent degradation for Basic Blue 26 irrespective of which manufacturer it is.

Figure 4.12 displays the aging curves for Basic Violet 3 and 1 present in the blue pens 1, 2, 3 and 4. Once again there was a fluctuating pattern between the different components with no simple successive degradation over time. Unlike with Basic Blue 26 in Figure 4.12, the lowest homologue m/z 288 in Basic Violet dyes was not the most abundant component produced during the degradation. This could suggest that the degree of degradation of Basic Blue 26 was greater than for Basic Violet dyes. The intermediate homologues; m/z 330, 344 were still being produced in higher quantities than m/z 288 for Basic Violet dyes.

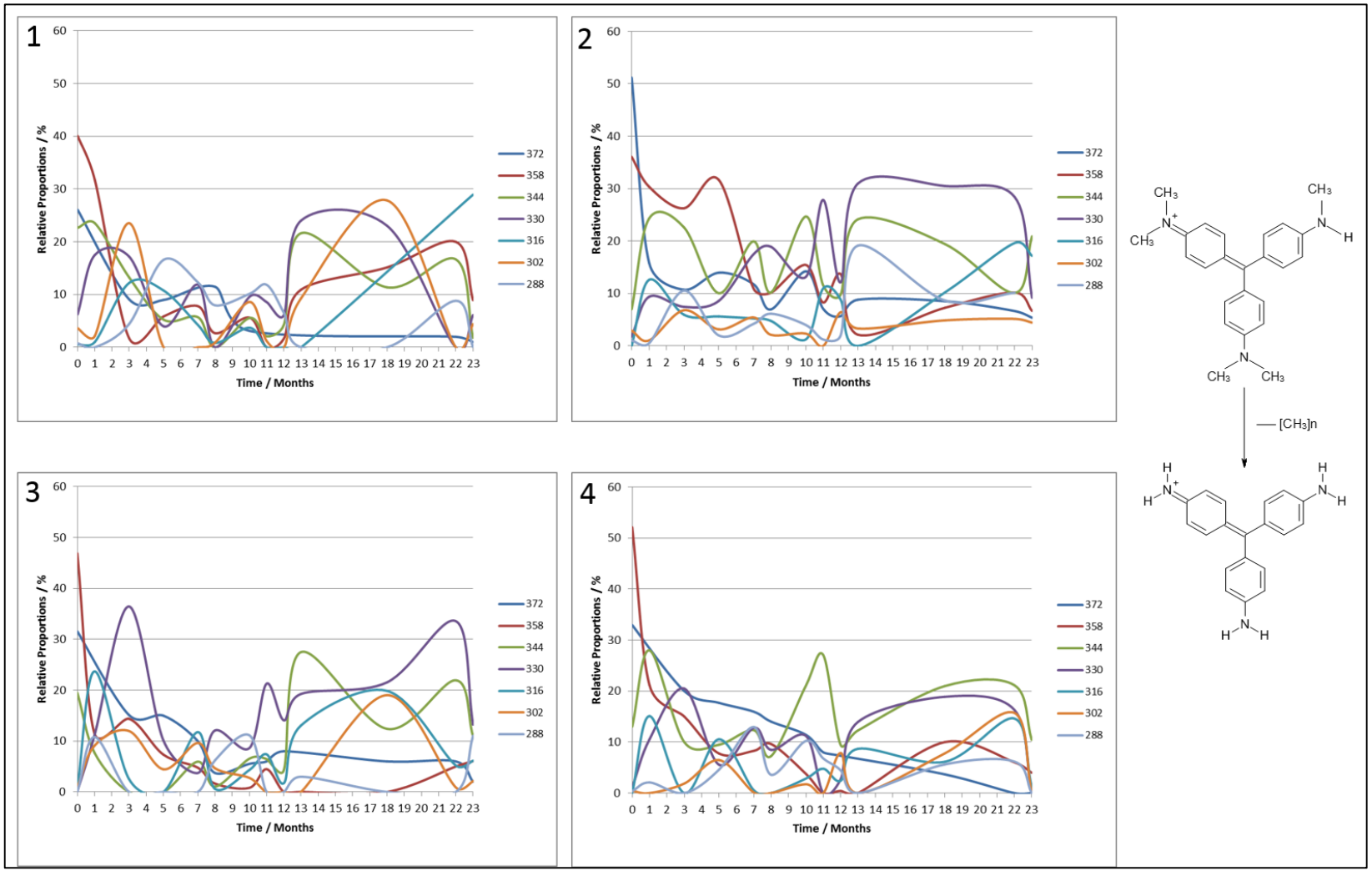


Figure 4.12 Aging Curves for Basic Violet 1 in Blue Pens 1: Micron 2: Bic 3: Staedtler, 4: Woolworths after 23 months exposure to sunlight

4.2.4 Comparison of Fading with No Light

As shown in previous sections, degradation occurs in the presence of light, but it could also occur without light. All ink on paper samples were stored in a folder and analysed in the same way as the light samples to determine if the light had an effect.

Figure 4.13 shows the direct comparison of Basic Blue 26 in pen 1 with and without light exposure over 23 months. These results were consistent across all blue pens and prove that light does indeed accelerate the degradation process.

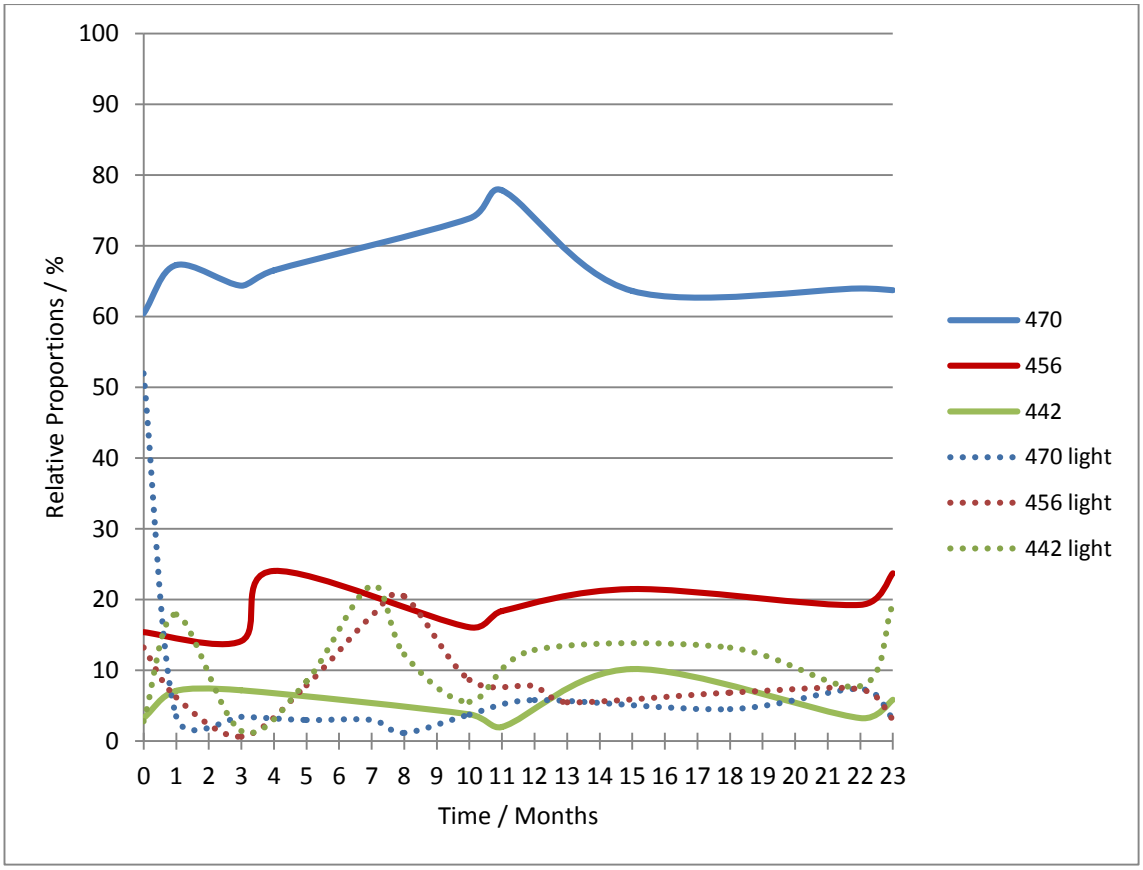


Figure 4.13 Aging Curves for Pen 1 – Basic Blue 26 with and without light exposure over 23 months

It is interesting to note that the light did not necessarily increase the values of the products formed, but they were produced much earlier.

In terms of ink dating, Figure 4.13 shows the importance of the storage conditions of the questioned document. If the storage conditions; temperature, humidity or light influence are unknown, the accuracy of ink dating is put into question.

4.3 Bleaching with Hydrogen Peroxide

4.3.1 Bleaching of Dye Standards

Each dye standard had 2 µl of 5% H_2O_2 added to it before being monitored by APCI-MS. Samples were kept in the dark or subjected to artificial daylight over six days to initiate the degradation process of bleaching.

4.3.1.1 In the Dark

The standard dye solutions were stored in a cupboard during this stage of analysis in order to be able to compare with samples subjected to artificial daylight. The aging curves for Basic Violet 1 and 3 are shown in Figure 4.14. For Basic Violet 3 (graph a) the molecular ion (m/z 372) decreased quickly to a relative proportion of 10% and the subsequent degradation homologue (mono-N-demethylated) m/z 358 increased. Similarly for Basic Violet 1, (graph b) m/z 358 decreased and the subsequent homologue m/z 344 increased. At T = 0 the relative ion count for the molecular ion m/z 372 was 100%, but at T = 6 days it had dropped to just 2.4% which was a significant decrease which could indicate a rapid degradation of the dye in just six days.

The degradation of Basic Violet 1 appears to be quicker than Basic Violet 3 as the dye homologues were being produced earlier and in greater quantity. At T = 0 the relative ion count for the molecular ion m/z 358 was 100% and at T = 6 days it had dropped to 16%. This was slightly higher than for Basic Violet 3 at 2.4%, but as Basic Violet 1 also contains some Basic Violet 3, during the demethylation process m/z 358 will be produced simultaneously by m/z 372 whilst it is also degrading.

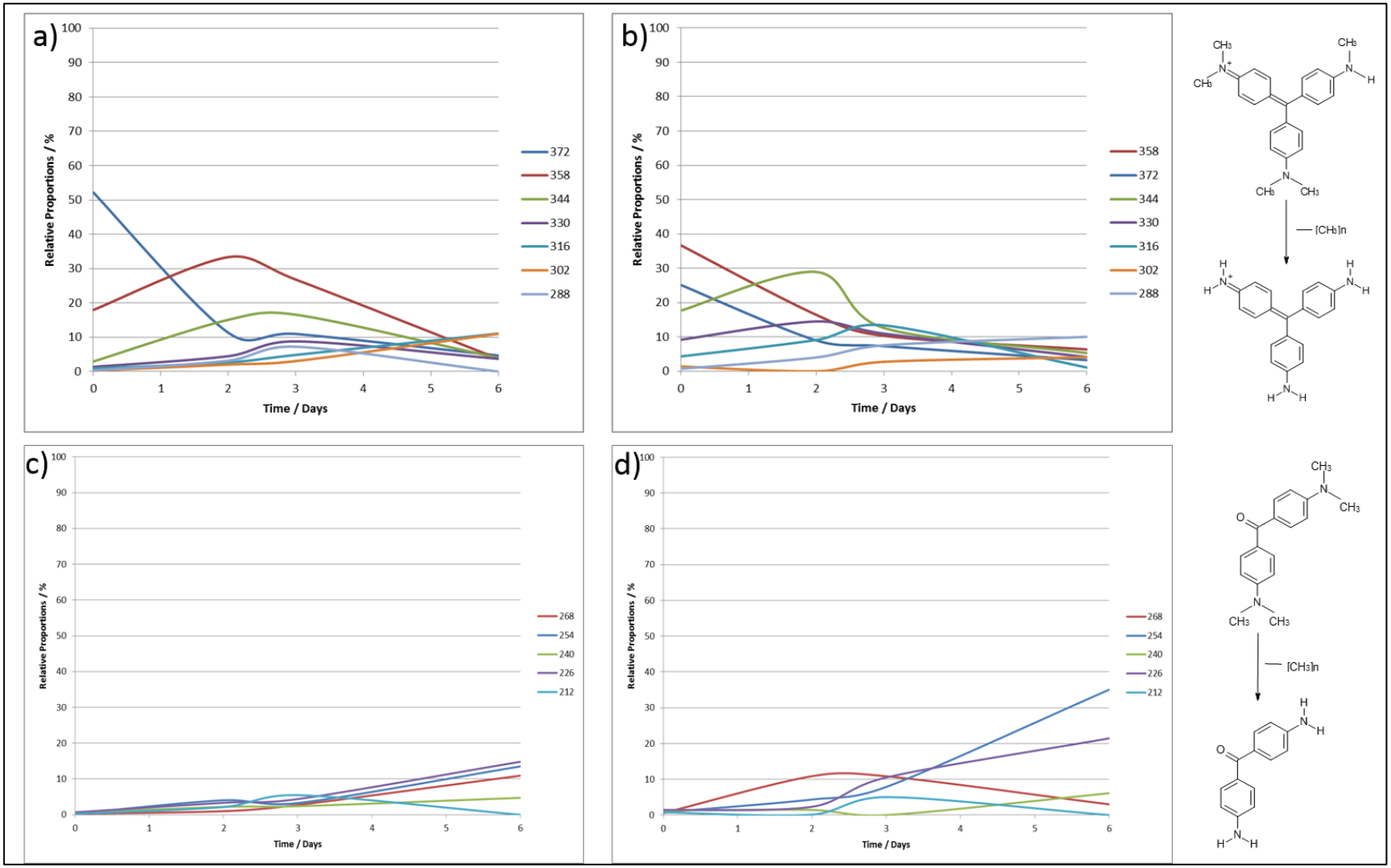


Figure 4.14 Aging Curves for dye standards a: Basic Violet 3 b: Basic Violet 1, c: Benzophenones for BV3 d: Benzophenones for BV1 during treatment with hydrogen peroxide

Benzophenone products were also detected after treatment with peroxide as shown in Figure 4.14 graphs c and d. In Basic Violet 3 all products except the fully demethylated product m/z 212 gradually increased over time. At T = 0 the relative ion count for the molecular ion m/z 268 was 0%, but at T = 6 days it had risen to 5%, however the predominant benzophenone at T= 6 days was m/z 226 with a value of 7.6%.

For Basic Violet 1 shown in graph d, m/z 254 was produced in a significant amount compared to the other products as the RP increased from 0 to 35%. This may be explained as it is formed from the loss of 1 -CH_3 group, which can be from either side of the molecule's structure which would double the possibility of it being produced. At T = 0 the relative ion count for the molecular ion m/z 268 was 0 and at T = 6 days was 7.6%. At T = 6 days the most abundant benzophenone was the tri-methylated ion m/z 254 with a value of 89%. These results are consistent with the rate of degradation being quicker for Basic Violet 1 than Basic Violet 3 as the values for the benzophenone products were higher.

The aging curves for Basic Blue 26 are displayed in Figure 4.15. The RP for molecular ion m/z 470 decreased from 90% to around 8% by three days where it remained, but the degradation homologues did not increase by significant numbers unlike for the Basic Violet dyes. In Basic Blue 26 the RP values never rose above 10% which would indicate limited formation of degradation products. At T = 0 the relative ion count for the molecular ion m/z 470 was 100% and at T = 6 days it had dropped to 12% which would indicate a quick reaction, but the homologues do not follow this pattern.
With regards to the benzophenone products, all RP values remained below 10% indicating limited degradation.

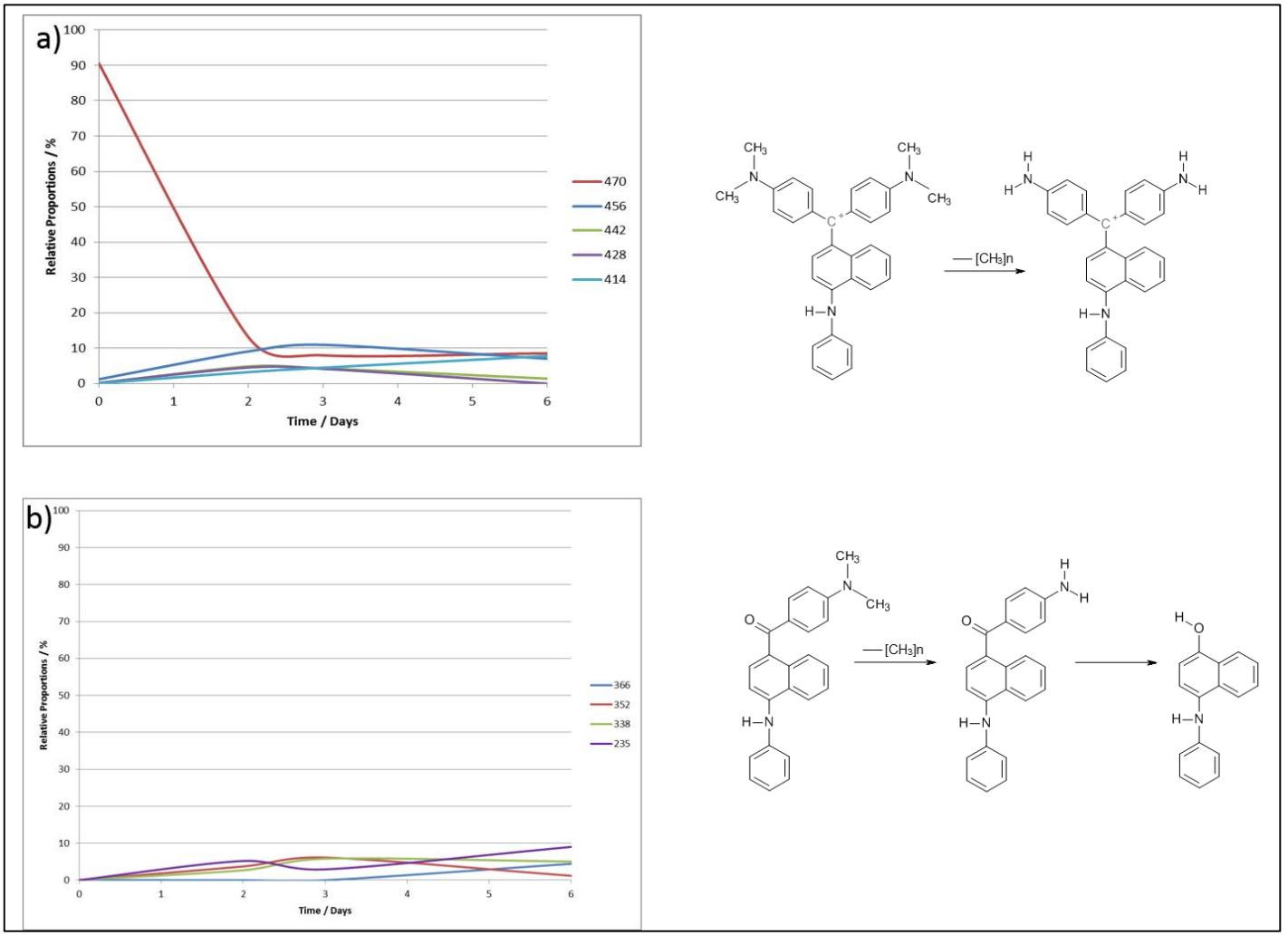


Figure 4.15 Aging Curves for Basic Blue 26 Aging Curves for dye standards a: Basic Blue 26 b: Benzophenones for BB26 during treatment with hydrogen peroxide

Phenol degradation products were detected for Basic Violet 3 from 2 days where the di-methylated product m/z 137 had a relative count of 7.5%. At six days m/z 137, 123 (mono-methylated) and 109 (fully demethylated) had relative counts around 3%. No phenol products were detected at six days for Basic Violet 1, but they were detected at two and three days with relative counts of 5% for m/z 137 and 2% for m/z 123. This would indicate that they were produced quickly, but were unstable and broke down. This would corroborate the theory of faster degradation of Basic Violet 1. Only m/z 235 was detected at six days for Basic Blue 26 with a relative count of 13%.

Table 4.7 shows the relative proportions based on ion count data of the component types when fresh and after six days. It clearly shows the reduction in dye components to at least half their original proportion and shows the increase in contribution of the degradation products; benzophenones and phenols, with the benzophenones most abundant after six days.

Table 4.7 Table to show the Relative Proportions of Products based on Ion Count Data after Treatment with Hydrogen Peroxide

	Basic Violet 3		**Basic Violet 1**		**Basic Blue 26**	
	T=0	**T=6**	**T=0**	**T=6**	**T=0**	**T=6**
Dye /%	74	38	77	35	90	25
Benzophenones /%	24	44	20	65	8	66
Phenols /%	2	17	2	0	1	9

Figures 4.14 and 4.15 have showed that even a small amount of hydrogen peroxide can induce aging in the dye standards and it was almost immediate as the effects were rapidly detectable with big changes occurring within two days. The values for m/z 372 and 358 in Basic Violet 3 and 1 had dropped significantly over the 6 days to only 5% and the lower homologues such as m/z 316 and 302 were already increasing at 2 days. There is published research on dye degradation through photochemical processes with the use of hydrogen peroxide,[65,152,153] but there is limited information on the effects without light. These results provide some evidence of de-methylation taking place without light. Benzophenone products were also produced for these dye standards providing

further evidence of degradation and more specifically of cleavage of the central carbon bond as described earlier. It is clear to see that hydrogen peroxide does have a role in the de-methylation process with or without light.

4.3.1.2 In the Light

In addition to the use of hydrogen peroxide to treat the dye standards, these samples were also subjected to artificial daylight (as detailed in Chapter 2) to see what affect it would have on the dyes. The results are shown in Figures 4.16 and 4.17. For the dye Basic Violet 3 in graph a, m/z 372 decreased quickly for two days from a relative proportion of 40% to around 5%. M/z 372 breaks down into m/z 358 which would explain why after three days the aging curve showed an increase for m/z 358. M/z 302 and 288 increased over the time period, with m/z 302 having the highest RP of 11% after six days. With just peroxide, m/z 302 also had the largest RP after 6 days and it was also 11%. M/z 330 and 344 did not show significant changes in RP remaining below 3%. Here m/z 316 increased to 11% at 3 days, but when just peroxide was used it did not reach this value until 6 days. At T = 0 the relative ion count for m/z 372 was 100%, but at T = 6 days it had dropped to 2%. All dye homologues were also low in value at six days, m/z 302 (penta-demethylated) was the most abundant increasing in ion count from 0 to 6%.

For Basic Violet 1, as shown in graph b, m/z 358 and 372 decreased sharply in RP for two days to around 5%. The later homologues m/z 330 (tri-methylated) and 316 (di-methylated) increased in RP for two days to peak at 11% and 16% respectively, but with light m/z 302 and 288 both showed significant increases in RP values (16% and 24% respectively) not seen without light. The most abundant component varied over time, but there was evidence of successive demethylation as m/z 316, then m/z 302 and finally m/z 288 were the most abundant one after the other. At T = 0 the relative ion count for the molecular ion m/z 358 was 100% and at T = 6 days it had decreased to 1.5%. The most abundant homologue at six days was the tri-demethylated product m/z 316 with an ion count of 7%.

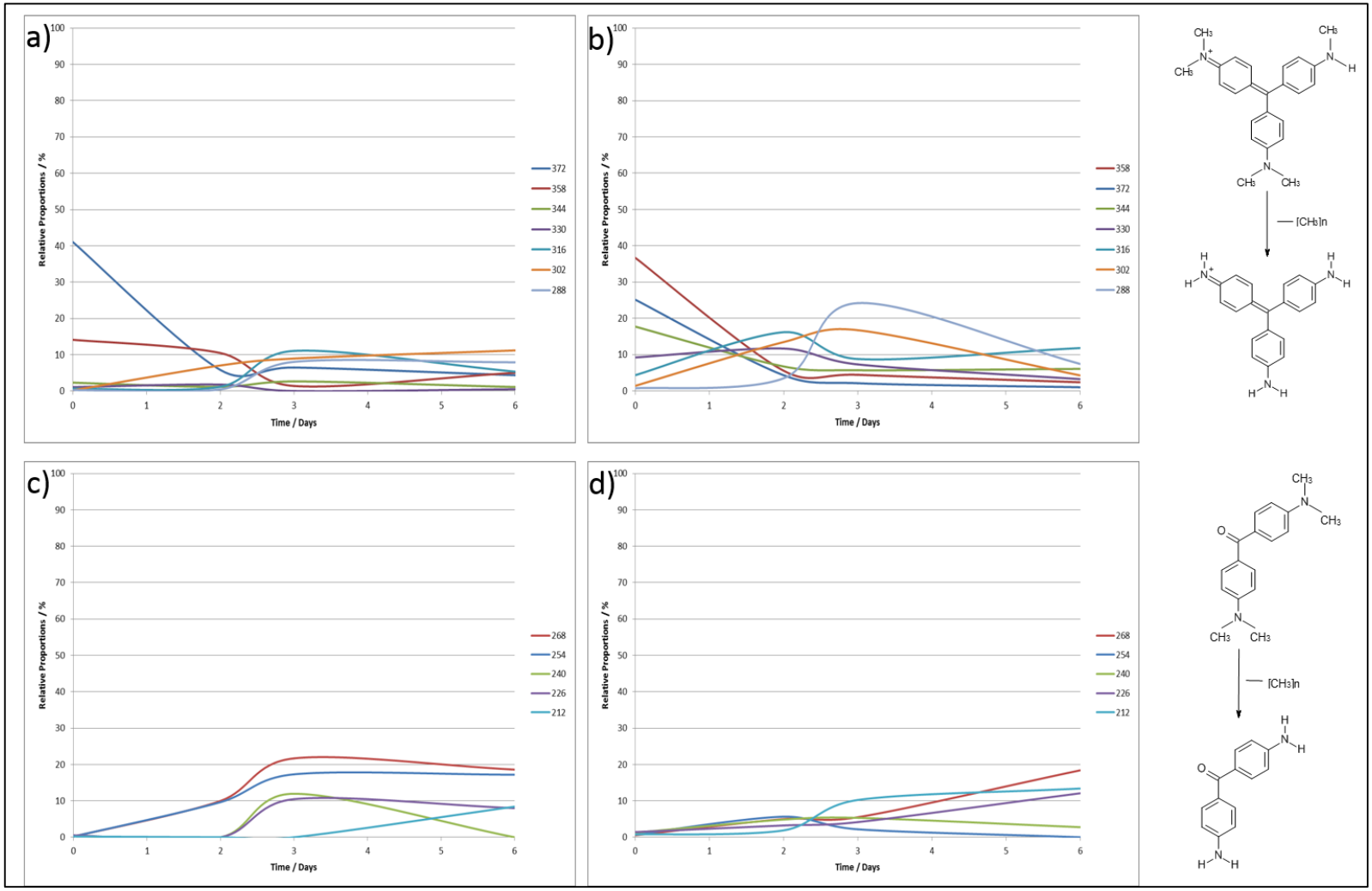


Figure 4.16 Aging Curves for dye standards a: Basic Violet 3 b: Basic Violet 1, c: Benzophenones for BV3 d: Benzophenones for BV1 during treatment with hydrogen peroxide and light

Benzophenone products were also produced under these conditions as shown in Figure 4.16 graphs c and d. With Basic Violet 3, the RP values for all products were greater than without light indicating that degradation is influenced by light and the breakage of the central carbon bond in the main dye structure does occur. At T = 0 the relative ion counts for the molecular ions m/z 268 and 254 were 0% and at T = 6 days they had risen to 10% and 9.5% respectively.

For Basic Violet 1, the RP values were lower with light than without light but at T = 0 the relative ion count for the molecular ion m/z 268 was 0% and at T = 6 days it had risen to 11%. However it was at T = 2 days that all benzophenone products had relative counts of between 8% and 78%, indicating they were produced early and after two days they were probably degrading themselves.

The aging curves for Basic Blue 26 following peroxide and light exposure are shown in Figure 4.17. M/z 470 decreased in relative proportion quite quickly and was almost at 0% by 2 days. The lower homologues m/z 442 and 428 had higher RP values with light than without, and m/z 456 did not seem to be part of the degradation process like with other conditions. It appears that the degradation products for Basic Blue 26 were not produced in the same proportions in relation to the main component m/z 470, compared to the products for the Basic Violet dyes in relation to m/z 372 and 358. At T = 0 the relative ion count for the molecular ion m/z 470 was 100% and at T = 6 days it was 0%. At six days, only two homologues were detected; m/z 456 and 428. This shows that the degradation of Basic Blue 26 was quick, but large quantities of degradation homologues were not produced during the process.

With the benzophenone products they behaved in the same way in that minimal quantities were produced even though degradation of the parent ion (m/z 470) had taken place. Total ion count data was well below 1% at 6 days for all benzophenone products. This would indicate that peroxide and light in combination do affect the degradation pathway of the dye standards and they do not follow the expected successive demethylation seen previously.

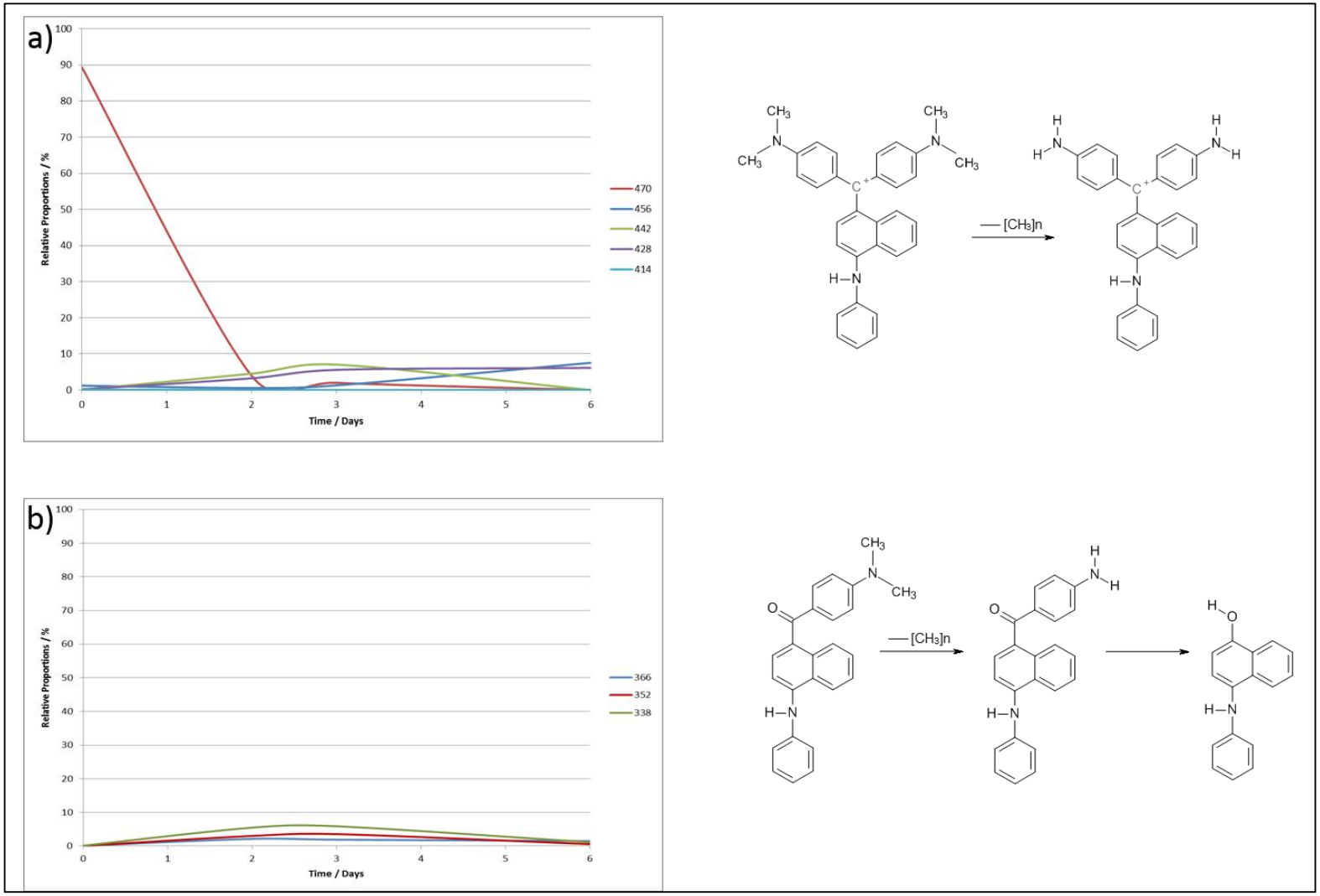


Figure 4.17 Aging Curves for dye standards a: Basic Blue 26 b: Benzophenones for BB26 during treatment with hydrogen peroxide and light

Phenol products were not detected until six days for Basic Violet 3; m/z 137 (di-methylated) and 109 both had relative counts of 3%. For Basic Violet 1, products were detected at 2 days. M/z 137 had a relative count of 9% which decreased to 2% at six days. For Basic Blue 26, phenol products were only detected at 2 and 3 days with relative counts for m/z 137 of 10 and 3%. These results indicate that phenol products were produced with peroxide and light, but in extremely low quantities.

Table 4.8 shows the relative proportions of the component types when fresh and after six days based on ion count data. It clearly shows the reduction in dye components which was greater than without light showing that light has enhanced the rate of degradation. The proportions of benzophenones and phenols have increased particularly for Basic Violet 1. Without light, no phenols were detected at six days, but with light the phenols contributed to 11% of the composition. The benzophenone values recorded were slightly less with light for Basic Violet 1, but this can be explained as they have degraded into the phenol products.

Table 4.8 Table to show the Relative Proportions of Products based on Ion Count Data after Treatment with Hydrogen Peroxide and Light

	Basic Violet 3		**Basic Violet 1**		**Basic Blue 26**	
	T=0	**T=6**	**T=0**	**T=6**	**T=0**	**T=6**
Dye /%	74	35	77	42	90	13
Benzophenones /%	24	52	20	46	8	81
Phenols /%	0	12	0	11	0	5

In order to determine if light in conjunction with peroxide has an impact on the degradation of the dye standards compared to no light exposure, comparative aging curves were produced and they are displayed in Figure 4.18. For all the blue dye standards, the relative proportion values were much lower for all components when light was used in combination with hydrogen peroxide which was not expected. It was anticipated that light would enhance the degradation and values would be higher. Without the light, increases in components were seen which would correspond to their successive production following the degradation of homologues.

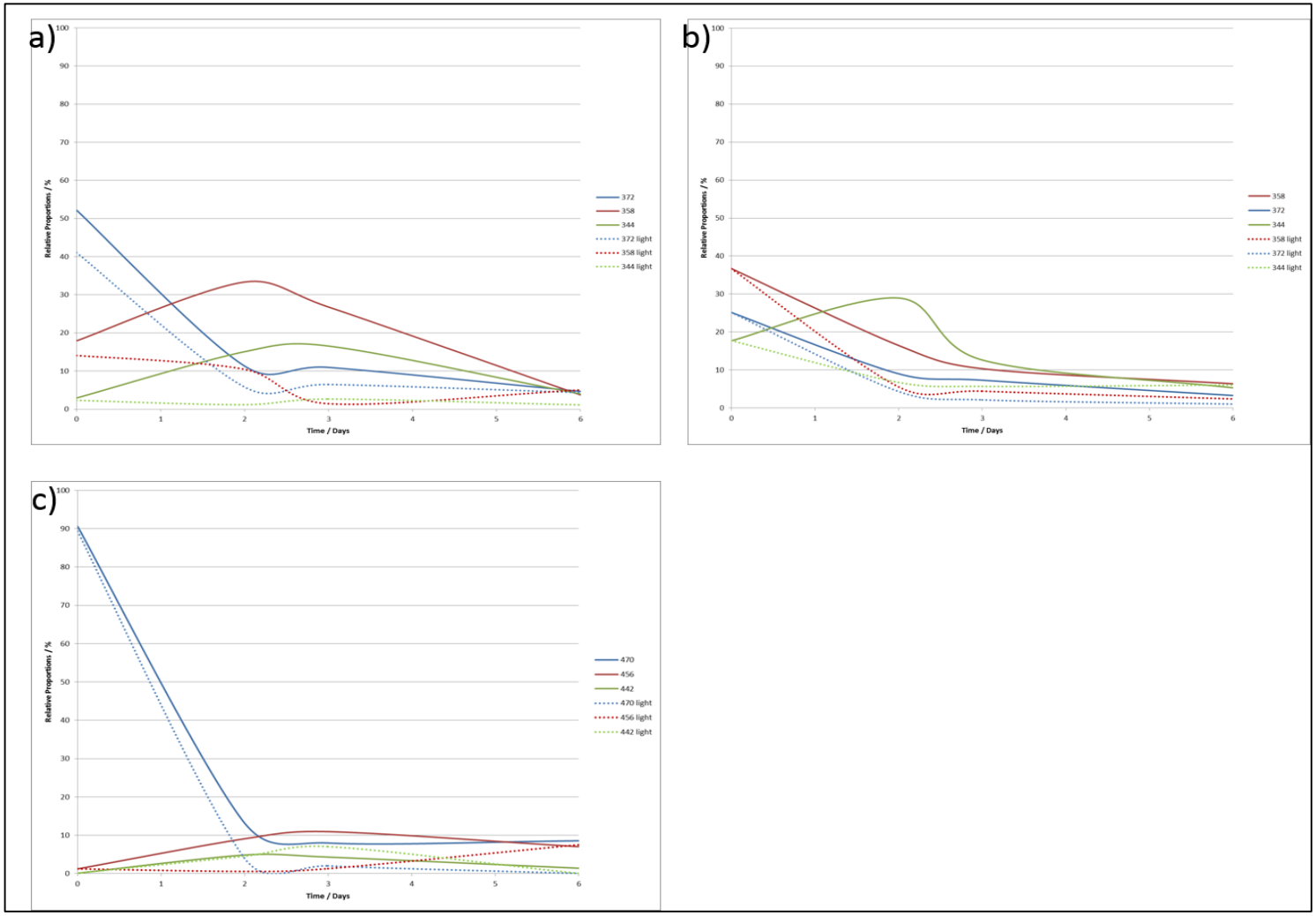


Figure 4.18 Aging curves for dye standards a: Basic Violet 3, b: Basic Violet 1 and c: Basic Blue 26 during Treatment with Hydrogen Peroxide with and without light

These results have shown that hydrogen peroxide has an impact on the degradation of dye compounds and in conjunction with light, the rate was quicker but the products were not formed in the same way or in the same proportions. It is important to find out if this will be the same when dyes are used in combination and when inks are on paper.

4.3.2 Bleaching of Dye Mixtures in Solution

By using dye combinations identified from the ink compositions of the ballpoint pen samples shown in Table 3.1, three dye standard combinations were produced as displayed in Table 4.9 for analysis to replicate ink degradation by hydrogen peroxide. APCI-MS analysis was carried out over ten days and aging curves produced of the results. Total ion counts were also used to determine the degree of fading.

Table 4.9 Dye Standard Mixtures

Dye Standard Mixture	Dye Standard Proportions	Pen Colour	Pen number
1	BV1 20 : BV3 15	Blue	2
2	BB26 100 : BV3 50	Blue	1
3	BV1 100 : BV3 70 : BB26 20	Blue	4

It can be seen in Figure 4.19 that for mixture 1, where there were two dyes of similar structure and therefore two dyes producing the same degradation products, that the RP values for the degradation homologues were higher than in mixture 2 where there was only one Basic Violet dye. M/z 344 was prominent in mixture 1, this is due to the loss of 2 $–CH_3$ groups from the structure, which could be from the same benzene ring or different rings, increasing the possibility of production. In mixture 2 the RP values for m/z 372 and 358 remained fairly consistent over the 10 days which could indicate little degradation. The RP values were also consistent for mixture 3 which contained 3 dyes. The aging curves were different to mixture 1, which would suggest that Basic Blue 26 influenced the behaviour of the Basic Violet dyes present, another observation of the synergistic fading effect of one dye on another.

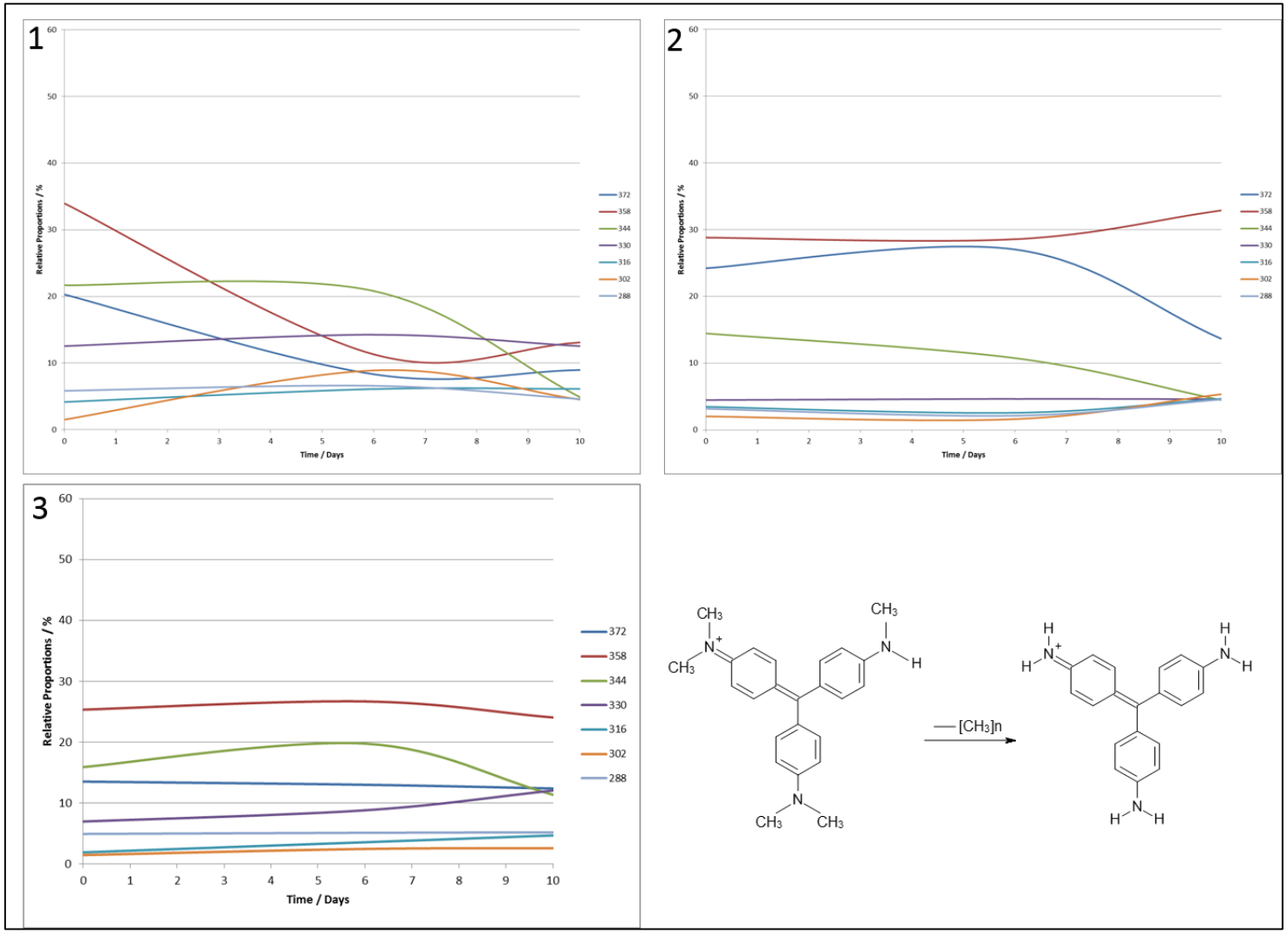


Figure 4.19 Aging Curves for Basic Violet Dyes in Dye Mixtures 1, 2 and 3 during Treatment with Hydrogen Peroxide

For mixture 1 at T = 0 the relative ion count for m/z 372 for Basic Violet 3 was 44% and 75% for Basic Violet 1. At T = 10 days m/z 372 had dropped to 13% and m/z 19% clearly showing degradation was occurring. For mixture 2, at T = 0 the ion counts were both 2%, at T = 10 days m/z 372 was still 2%, but m/z 358 had increased to 5%. (The low values can be attributed to the original composition whereby Basic Blue 26 is the main component). These values indicate little degradation of the Basic Violet dyes. For mixture 3, the ion counts for both m/z 372 and m/z 358 remained the same after 10 days at 25% and 48% respectively, indicating little degradation.

Figure 4.20 displays the aging curves for Basic Blue 26 in mixtures 2 and 3. Basic Blue 26 in mixture 2 (graph a) maintained a high RP value of around 90%. The lack of degradation was seen in the relative ion count data as well. At T = 0 m/z 470 was 100% and at T = 10 days it was still 100%. In mixture 3 which contained less Basic Blue 26 than in mixture 2, the dye decreased in proportion steadily from the beginning of the treatment with increased production of the primary degradation products. At T = 0 m/z 470 was 76% and at T = 10 days it was 18%. This would suggest that when Basic Blue 26 is in large quantities it is extremely stable, but when in small quantities it is vulnerable to degradation by hydrogen peroxide.

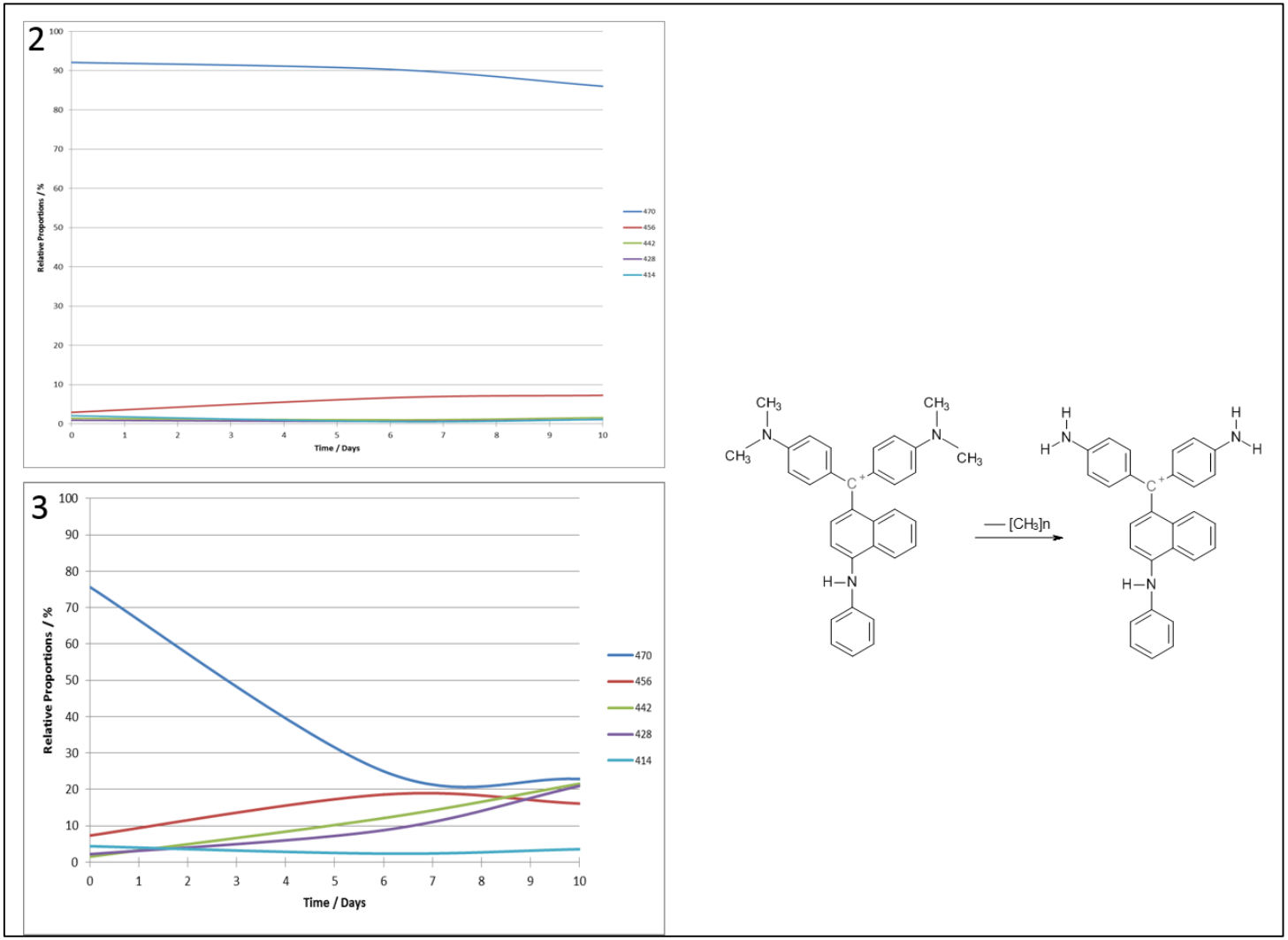


Figure 4.20 Aging Curves for Basic Blue 26 in Dye Mixtures 2 and 3 during Treatment with Hydrogen Peroxide

The benzophenone products for dye mixtures 1, 2 and 3 are displayed in Figure 4.21. All RP values were below 15% which would suggest minimal degradation occurring which was consistent with the parent dye data. For mixture 1, m/z 254 and m/z 226 had the highest values which could correspond to two dyes producing the same degradation products, but for the same dyes in mixture 3, the homologues were not produced until days later. This would suggest that Basic Blue 26 which was present in mixture 3 was influencing the degradation of the Basic Violet dyes and potentially making it slower. In mixture 2 where Basic Blue 26 was also present alongside Basic Violet 3, the products from Basic Violet 3 were produced earlier than in mixture 3 indicating faster degradation when Basic Blue 26 is present in high quantities. This would imply there could be competition between the different dyes for the hydrogen peroxide.

The benzophenone products specifically from Basic Blue 26 were not detected in high enough quantities to confirm their presence. The relative count data supports this as no values were higher than 2%, indicating they were not produced in large quantities if at all.

It appears that for the mixtures and hydrogen peroxide, the effects were slightly different to photofading, especially for Basic Blue 26. When there was a small amount of this dye as in mixture 3, its degradation appeared to be greater than the degradation in mixture 2 for large amounts of the dye, unlike with photofading when in either condition the dye appeared stable.

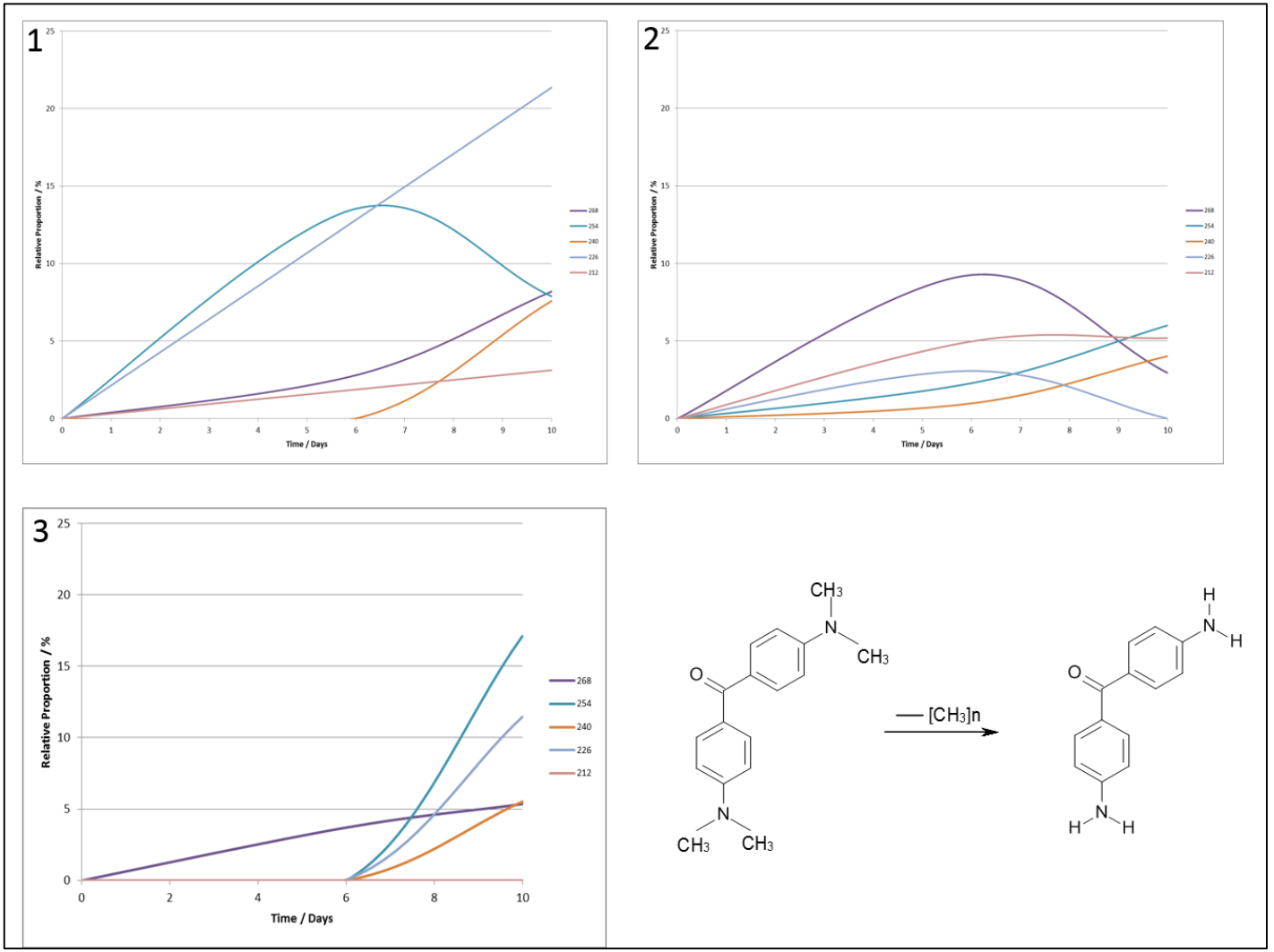


Figure 4.21 Aging Curves for Benzophenone Products from Basic Violet 3 in Dye Mixtures 1, 2 and 3 during Treatment with Hydrogen Peroxide

Table 4.10 shows the relative proportions of the component types in the dye mixtures when fresh and after 10 days based on ion count data. The reduction in the dye components over time can be seen, but the amount of change was different for each mixture with mixture 1 having the greatest reduction in dyes, and mixture 2 the greatest increase in benzophenones.

Table 4.10 Table to show the Relative Proportions of Products based on Ion Count Data in Dye Mixtures after Treatment with Hydrogen Peroxide

	Dyes /%		Benzophenones /%		Phenols /%	
Mixture	**T = 0**	**T = 10**	**T = 0**	**T = 10**	**T = 0**	**T = 10**
1	80	54	20	45	0	1
2	97	77	1	18	0	5
3	80	75	17	24	0	1

4.3.3 Bleaching of Inks on Paper

Hydrogen peroxide was applied to the ink samples when written on the paper surface. Figure 4.22 show the visible light results after three applications of hydrogen peroxide to all samples.

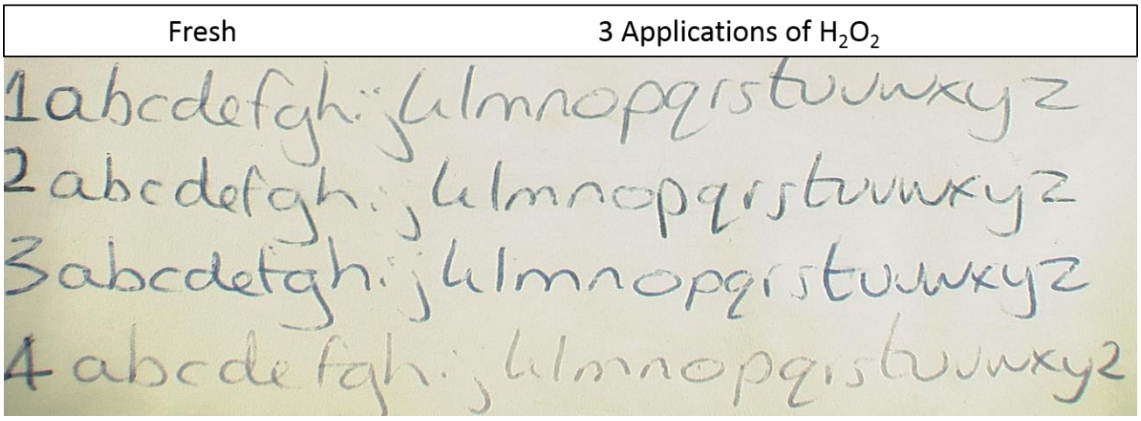


Figure 4.22 Image of Fresh Blue Ink and Ink treated with 10 applications of Hydrogen Peroxide

After one application of peroxide, the inks were slightly lighter than without, but no significant fading had occurred. Peroxide was applied to the ink samples a total of ten times allowing them to dry between each application. Fading had occurred for all samples, but not to the same extent for each other. Sample 4 (Woolworths) showed the most significant fading with little colour remaining. Sample 2 (Bic) had changed colour to a turquoise shade which is not seen in any

other blue inks. This has been recorded in the literature for some blue inks subjected to light,[70] but no information has been found relating to inks and peroxide bleaching.

Fluorescence was also examined for the peroxide samples and the images are shown in Figure 4.23. Samples 3 and 4 (Staedtler and Woolworths) exhibited fluorescence with and without peroxide treatment, the Micron and Bic samples did not fluoresce before or after ten applications of peroxide treatment.

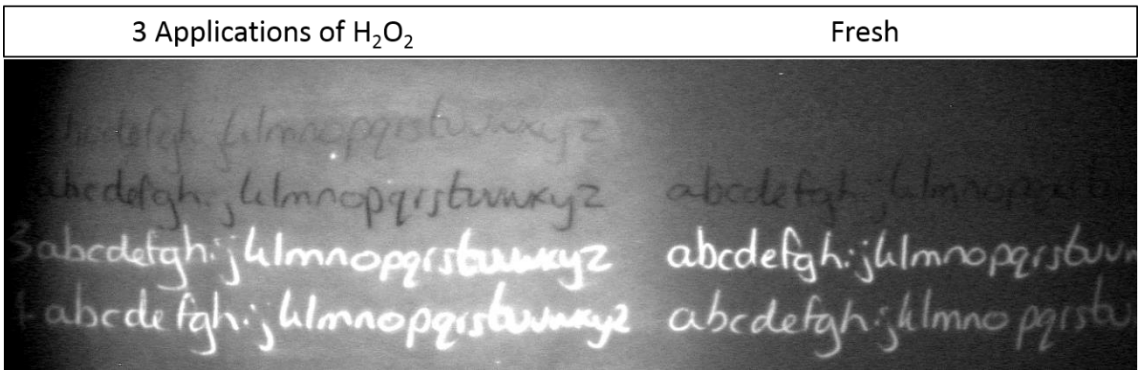


Figure 4.23 VSC Fluorescence of Blue Pen Samples Fresh and after 3 applications of Hydrogen Peroxide

These results were consistent with the results from photofading whereby samples 3 and 4 fluoresced pre and post treatment, and samples 1 and 2 did not which may be attributed to the presence of Basic Blue 26 but further investigation is required.

Ink samples were analysed by APCI-MS over 10 applications. Figure 4.24 displays the aging curves for the dye Basic Blue 26 which is one of the dyes present in pens 1, 3 and 4, Micron, Staedtler and Woolworths respectively. Visually Staedtler was the least faded of the blue samples and Woolworths was the most faded, and this was supported by the aging curves. For Staedtler the relative proportion for m/z 470 remained between 60% - 75% during the treatment and the degradation homologues did not increase significantly in their values, which could indicate there was little degradation occurring, whereas for Woolworths the RP values for all dye components changed significantly more over the course of the treatment. M/z 470 decreased from 50% to 10% and the other components increased in RP, particularly m/z 414. As m/z 470 was still detected for all pens, it would suggest that degradation was far from complete.

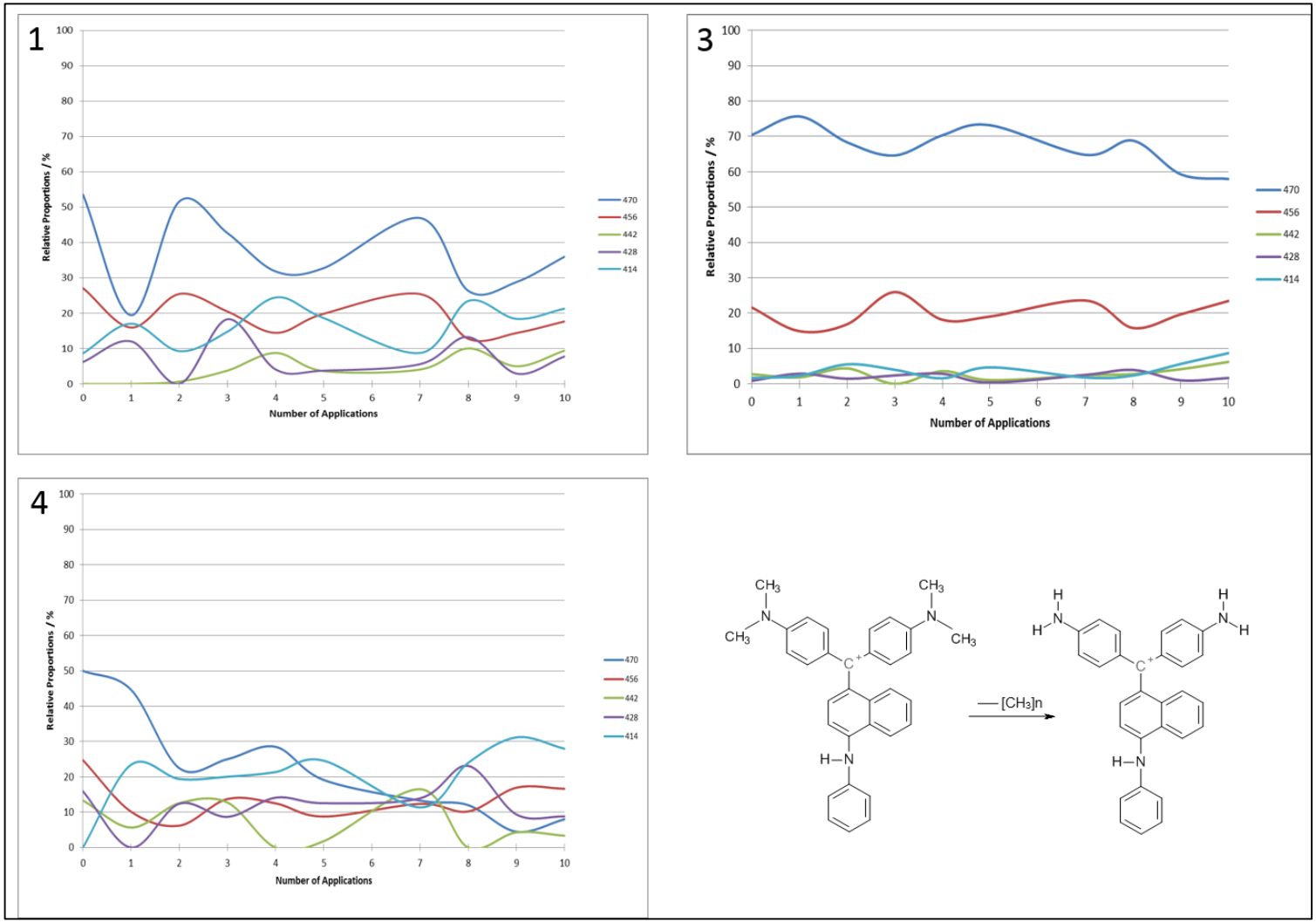


Figure 4.24 Aging Curves for Basic Blue 26 in Blue Pens 1: Micron, 3: Staedtler, 4: Woolworths after treatment with hydrogen peroxide

The aging curves for the Basic Violet 3 and 1 in pens 1, 2, 3 and 4 can be seen in Figure 4.25. It was clear again that the degradation for Staedtler was less than for Woolworths. Fewer degradation homologues have been produced and the relative proportions of m/z 372 and m/z 358 were clearly higher than the other components. For Woolworths, the parent dyes decreased rapidly in RP values and was already half its starting RP by three applications of peroxide. The RP values for the homologues for Woolworths were much more varied and generally increased in value during the treatments as degradation increased. At T = 0 the relative count for m/z 372 in Staedtler was 85% and in Woolworths was 100%. At T = 10 applications, the counts were 23% for Staedtler and 7% for Woolworths showing increased degradation for the Woolworths ink. Based on the aging curves, the Bic pen was similar to Staedtler in that RP values were consistent, but Micron was more similar to Woolworths with increased activity by the homologues.

Discrimination between the pens was clear based on the aging curves and degradation of Basic Blue 26. Definitive discrimination appears more difficult based on Basic Violet data.

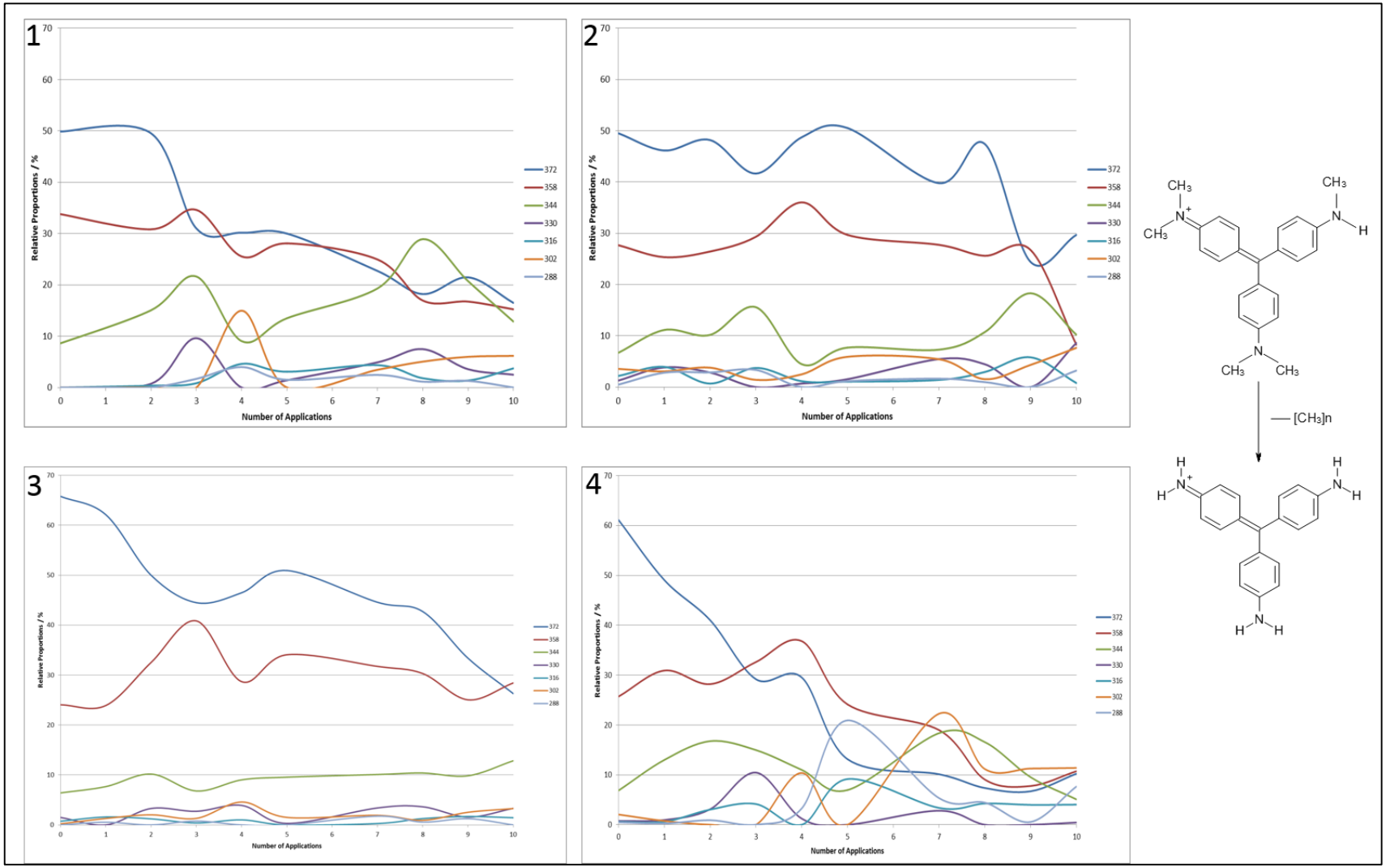


Figure 4.25 Aging Curves for Basic Violet 1 in Blue Pens 1: Micron, 2: Bic, 3: Staedtler, 4: Woolworths after treatment with hydrogen peroxide

Benzophenone products were detected for these samples and the aging curves are displayed in Figures 4.26 and 4.27. As there was more visual fading for ink 4 than ink 3, and therefore more degradation for ink 3, it would be expected that the proportions would be more for the benzophenone products produced, so higher RP values for ink 3 compared to ink 4. This was clear to see in the aging curves. There was much less activity for ink 4 and it occurred later than ink 3. Ink 1 also displayed more benzophenone activity than ink 2 which corresponds with the parent dye data in Figure 4.25. This was also seen for the degradation products of Basic Blue 26 in inks 1, 3 and 4 shown in Figure 4.27. Higher RP values (18%) were observed for pen 4 compared to values of 3% for pen 3, but also high values for pen 1 like with the parent dye data.

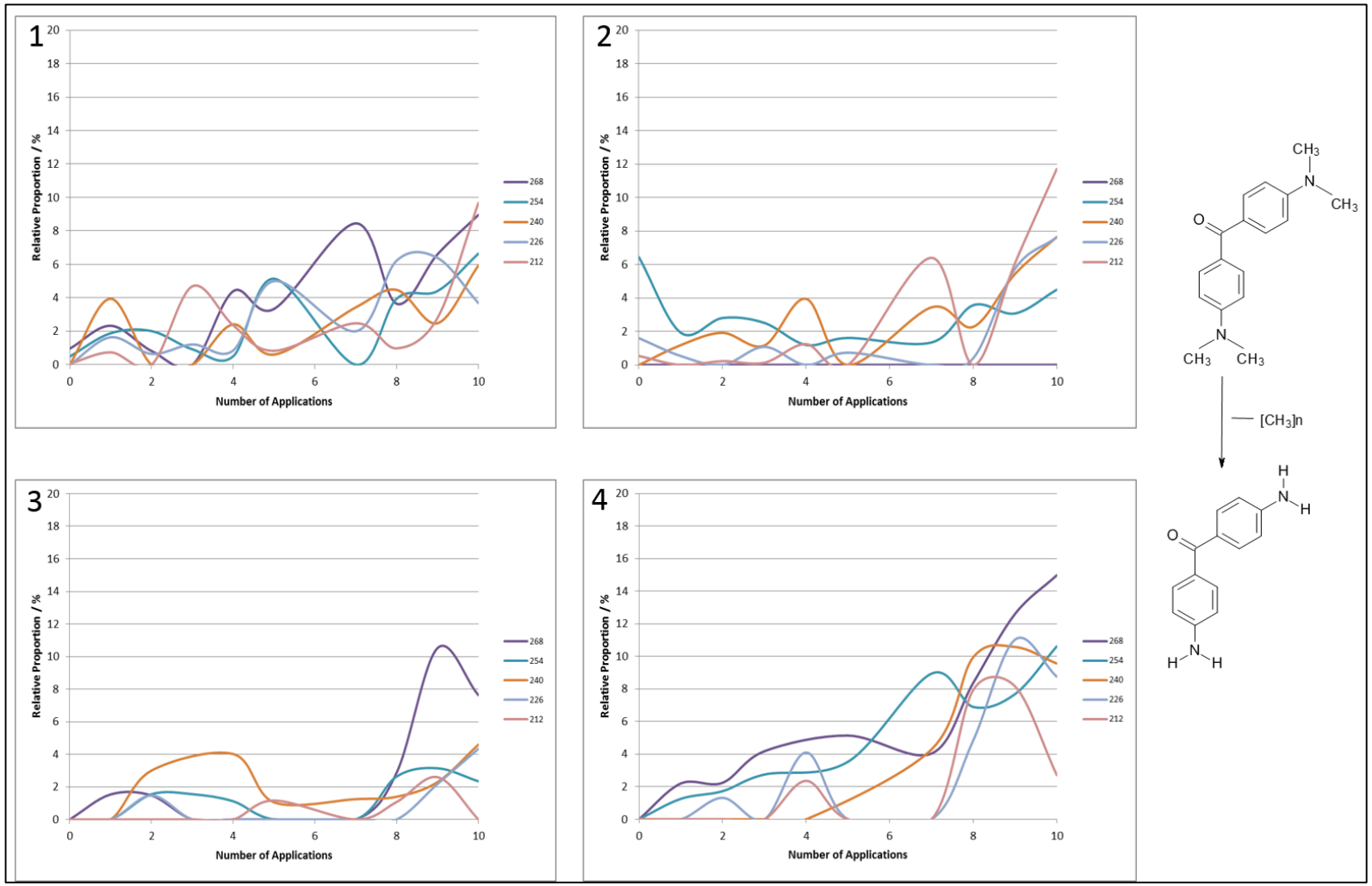


Figure 4.26 Aging Curves for Benzophenone Products for Basic Violet 3 in Blue Pens 1: Micron, 2: Bic, 3: Staedtler, 4:Woolworths after treatment with hydrogen peroxide

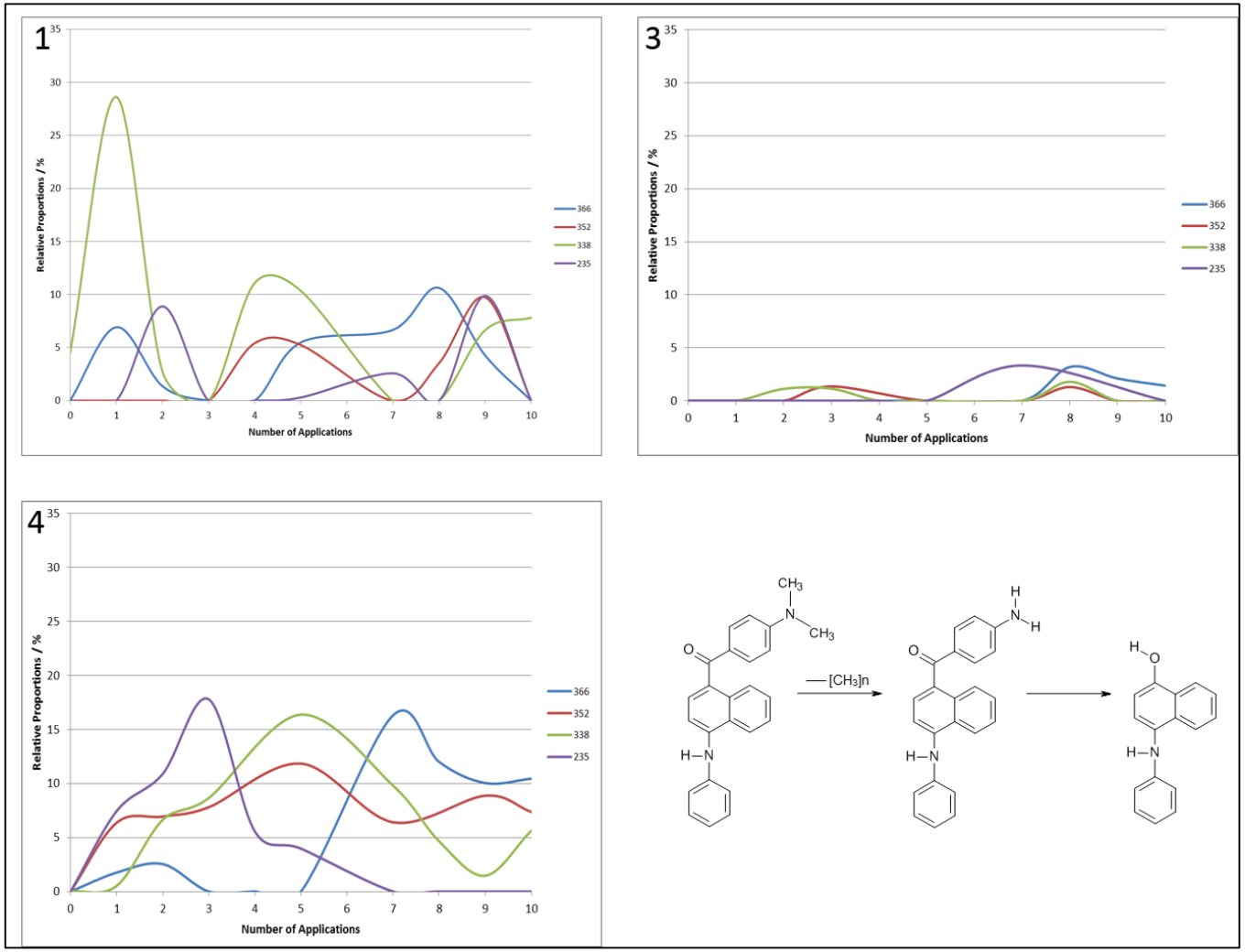


Figure 4.27 Aging Curves for Benzophenone Products for Basic Blue 26 in Blue Pens 1: Micron, 3: Staedtler, 4: Woolworths after treatment with hydrogen peroxide

It can be seen that discrimination between the pens was possible based on APCI-MS data and aging curves and that hydrogen peroxide has influenced the fading of the inks, but even though inks may contain the same dyes, the degree of degradation was not consistent between samples.

4.4 Bleaching with Sodium Hypochlorite

4.4.1 Bleaching of Dye Standards

Each dye standard had 2 µl of 5% household bleach added to it before being monitored by APCI-MS. Samples were kept in the dark or subjected to artificial daylight over a period of time to initiate the degradation process of bleaching. Analysis was carried out immediately after treatment then after 2, 17, 28 and 40 days.

4.4.1.1 In the Dark

The standard dye solutions were stored in a cupboard during this stage of analysis in order to be able to compare with samples subjected to artificial daylight. The mass spectra for some of the dyes are shown in Figures 4.28-4.30.

Figure 4.28 shows the mass spectra for Basic Violet 3 after treatment with 5% bleach. As expected, the peak for the molecular ion m/z 372 was most abundant with m/z 358 (mono-N-demethylated) slightly smaller. By 17 days, m/z 358 was more prominent than m/z 372 and remained so for the whole time period. Also at this time the other demethylation products m/z 344 and 330 started to increase. At 40 days all previous components were still present with m/z 358 the highest intensity. Here m/z 330 had increased and was greater than 344. There was evidence of benzophenones present, as there was a peak visible at m/z 268 which corresponds to di-methyl, di-methylaminobenzophenone. These results show the degradation of Basic Violet 3 by bleach over time by demethylation and breakage of the central bond.

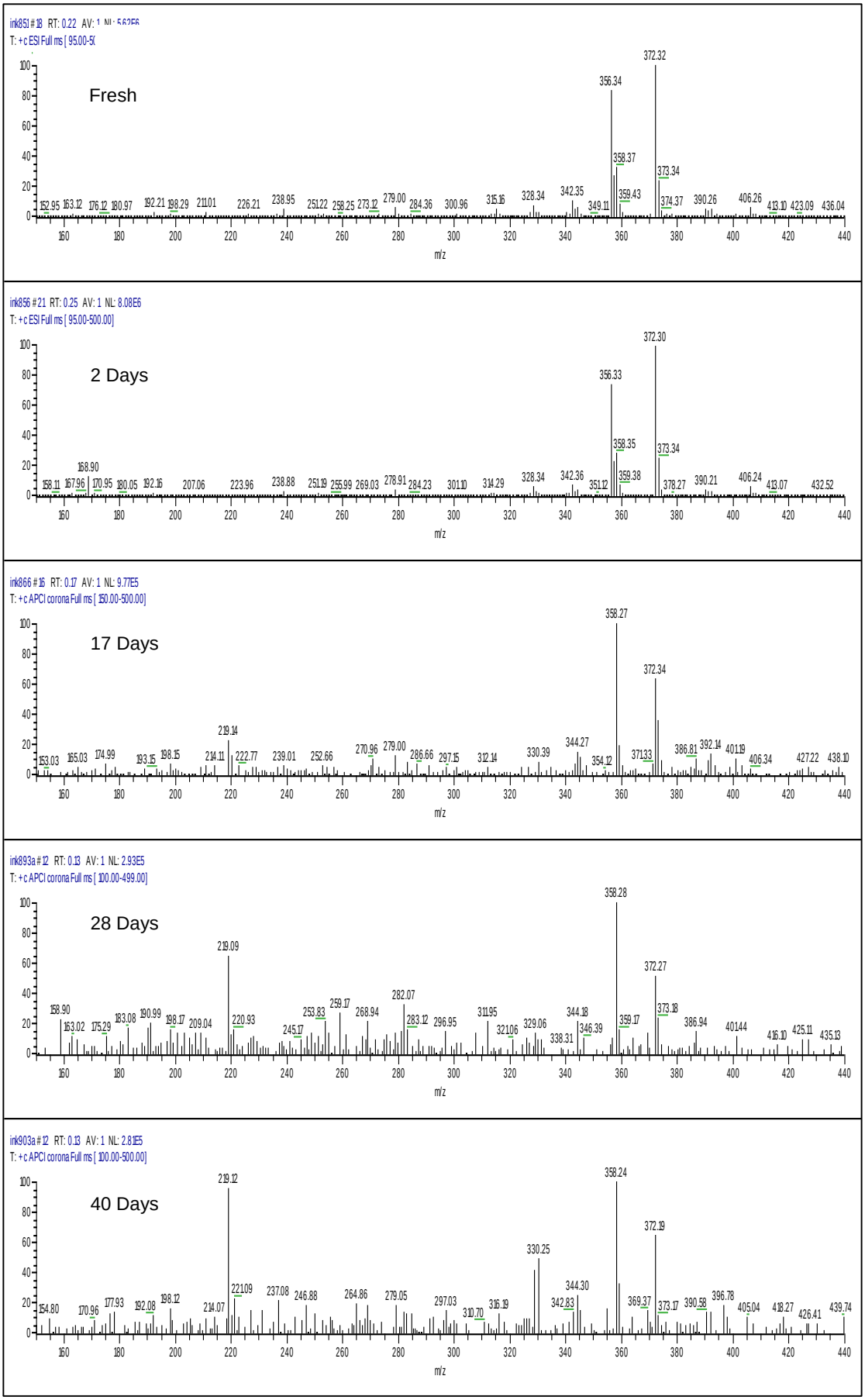


Figure 4.28 APCI-MS Spectra for Basic Violet 3 during Treatment with 5% Bleach

Basic Violet 1 also degraded with bleach as illustrated in Figure 4.29. Peaks at m/z 358, 372, 344 and 330 were all clear to see in the fresh sample, with m/z 358 as expected, the most abundant, followed by m/z 372. After 2 days there were few visible differences, however by 17 days, all the dye peaks were significantly smaller, which was attributed to the contaminant peak at m/z 270; however they can still be identified and m/z 358 was still the most abundant. By 40 days, the dye peaks were not clearly visible so the actual MS data was needed to determine if they were still present. Basic Violet 1 appeared to follow the same demethylation process of degradation as Basic Violet 3. The peak visible at m/z 219 was not a result of degradation, but from contamination within the equipment.

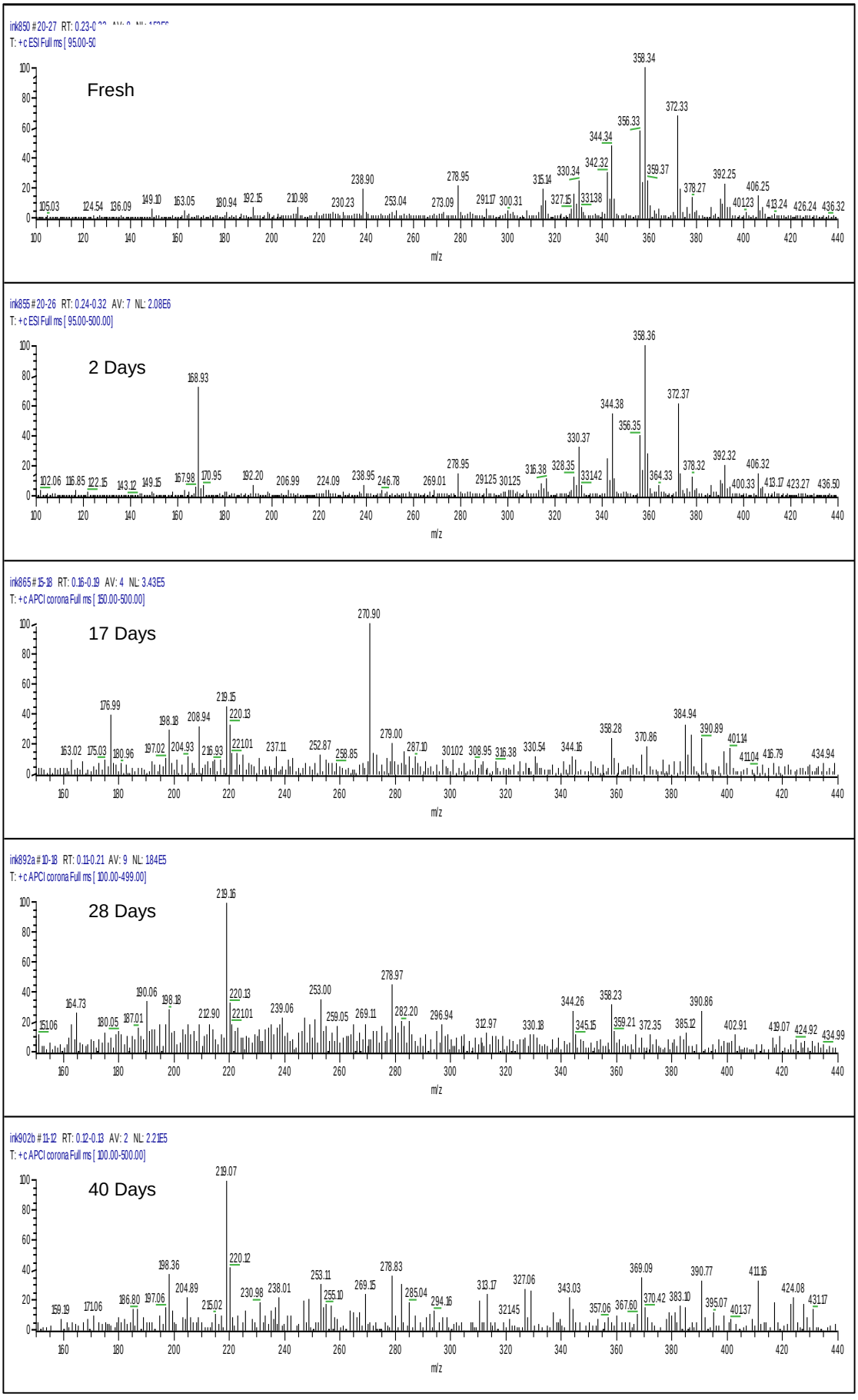


Figure 4.29 APCI-MS Spectra for Basic Violet 1 during Treatment with 5% Bleach

Basic Blue 26 appeared to be a stable dye, as Figure 4.30 indicates. Even after 40 days, m/z 470 was still the most abundant peak, with few other peaks visible. After 17 days, a peak at m/z 456 (mono-demethylated) appeared and became gradually larger up to 40 days, but no other peaks were identified. Basic Blue 26 has started to follow the demethylation degradation pathway as in the literature, i.e. the loss of a methyl group, but further degradation was needed.

The dyes did change visually over the 40 days becoming much lighter in colour, however, none of the standards became colourless during this time.

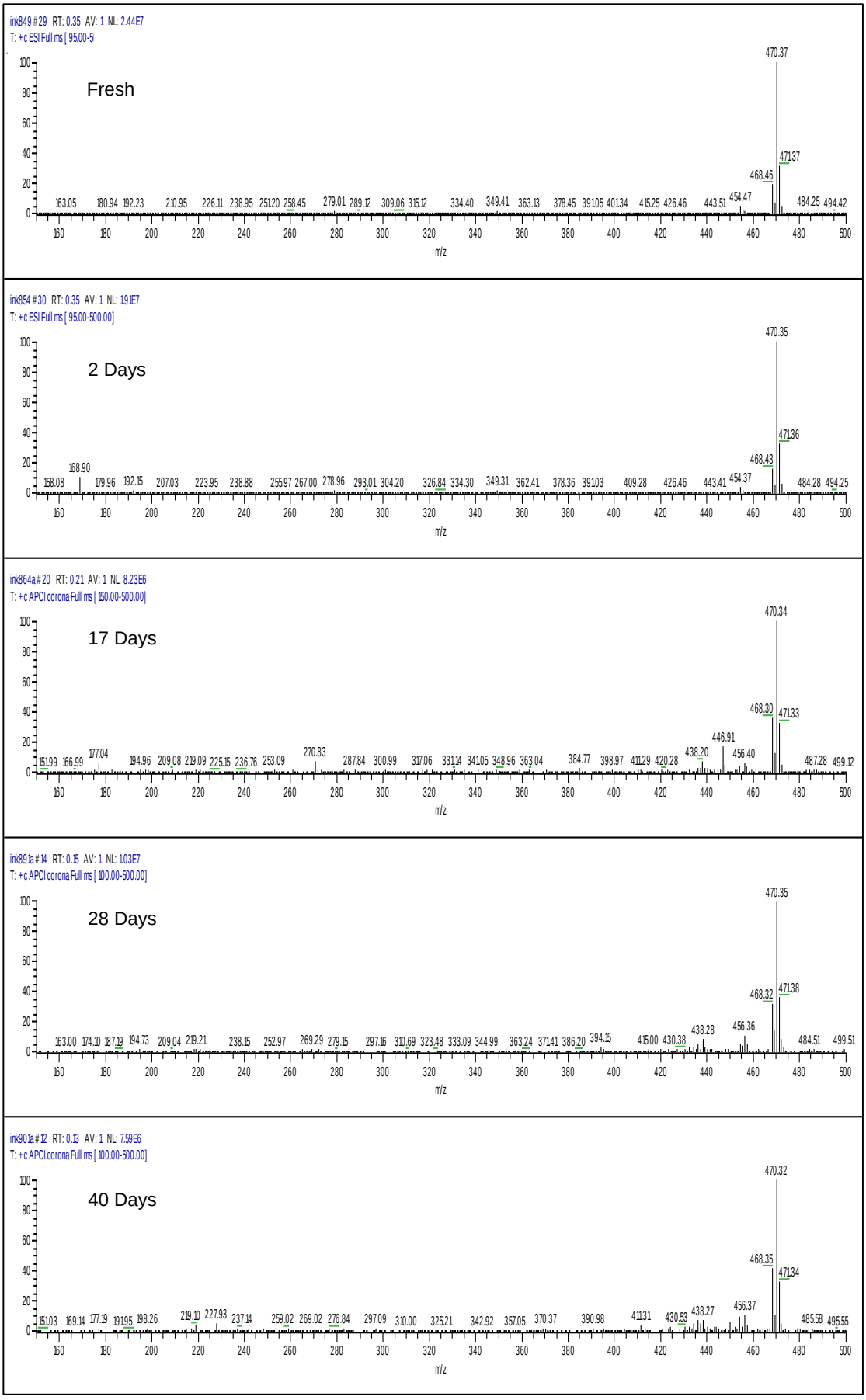


Figure 4.30 APCI-MS Spectra for Basic Blue 26 during Treatment with 5% Bleach

Using the data from the mass spectra, aging curves were created and Figure 4.31 shows the changes that occurred for Basic Violet 3 and 1 over the 40 days.

As seen in the mass spectra and as shown in the aging curve, both parent dyes decreased in RP over the 40 days, and the homologues increased suggesting degradation was taking place due to bleach. This was supported by the ion count data. For Basic Violet 3 at T = 0 m/z 372 was 100% and dropped to 42% at T = 40 days and for Basic Violet 1 m/z 358 dropped from 100% to 6%. This also supports previous evidence that more degradation has occurred for Basic Violet 1 than Basic Violet 3.

There were still coloured components detectable and visible after 40 days, as the bleach had not completely decolourised the Basic Violet 3 solution.

As well as demethylation and deethylation, cleavage of the central phenyl bond can occur which produces benzophenone products. Graphs c and d in Figure 4.31 show the aging curves for these products for Basic Violet 3 and 1 after treatment with 5% bleach. The RP's were not particularly high, none were above 4% for Basic Violet 3, but it does indicate that benzophenone products were produced, as the proportions increased over the 40 days. For basic Violet 1, the RP values were higher for all products supporting the theory that more degradation takes place for this dye than Basic Violet 3.

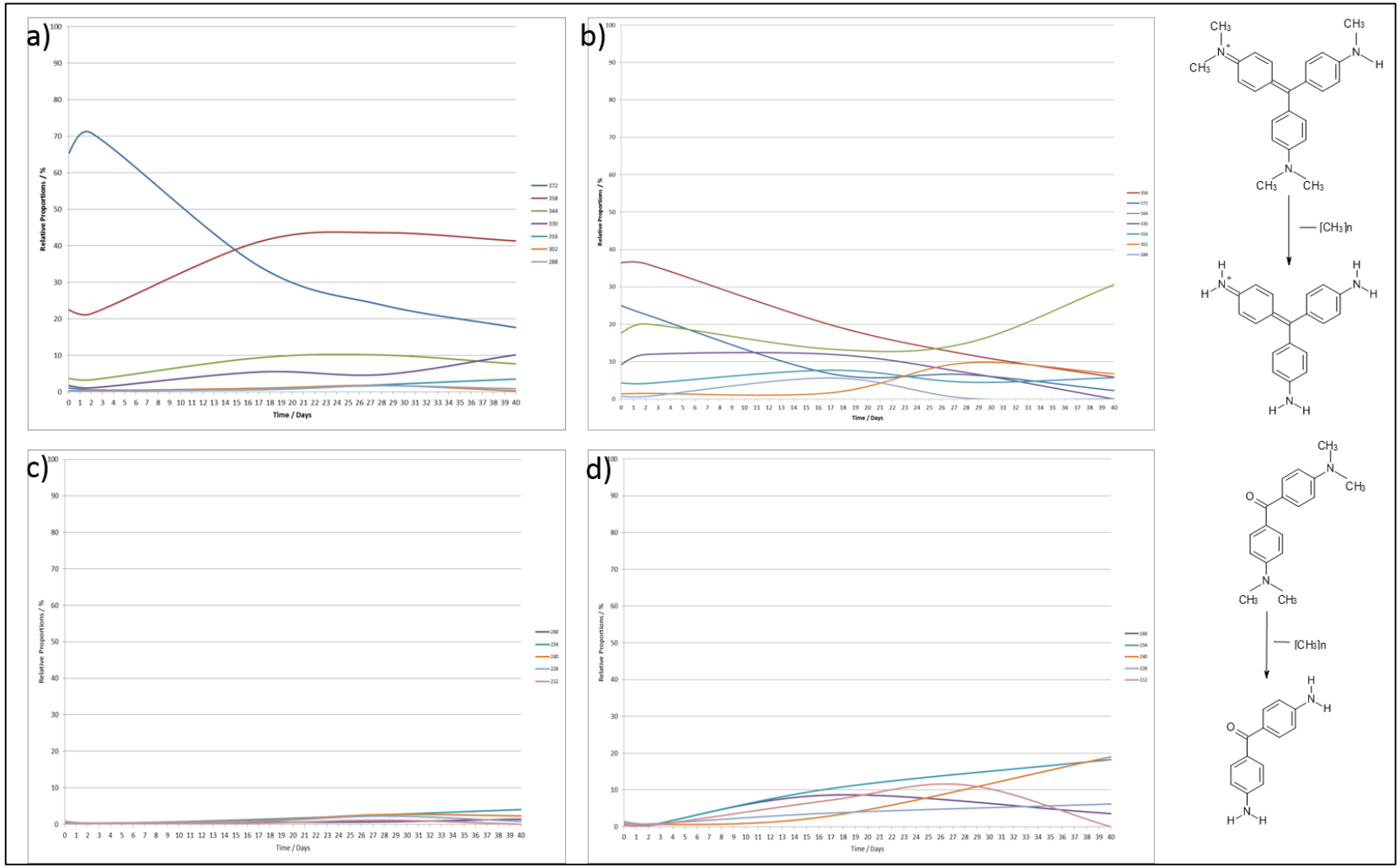


Figure 4.31 Aging Curves for dye standards a: Basic Violet 3, b: Basic Violet 1, c: Benzophenones for BV3, d: Benzophenones for BV1 during treatment with 5% bleach

Figure 4.32 displays the aging curves for both dye components. It can be clearly seen that Basic Violet 1 produced benzophenone degradation products more quickly than Basic Violet 3 indicating that Basic Violet 3 was more stable than Basic Violet 1. These results are consistent with previous results from photofading and peroxide bleaching.

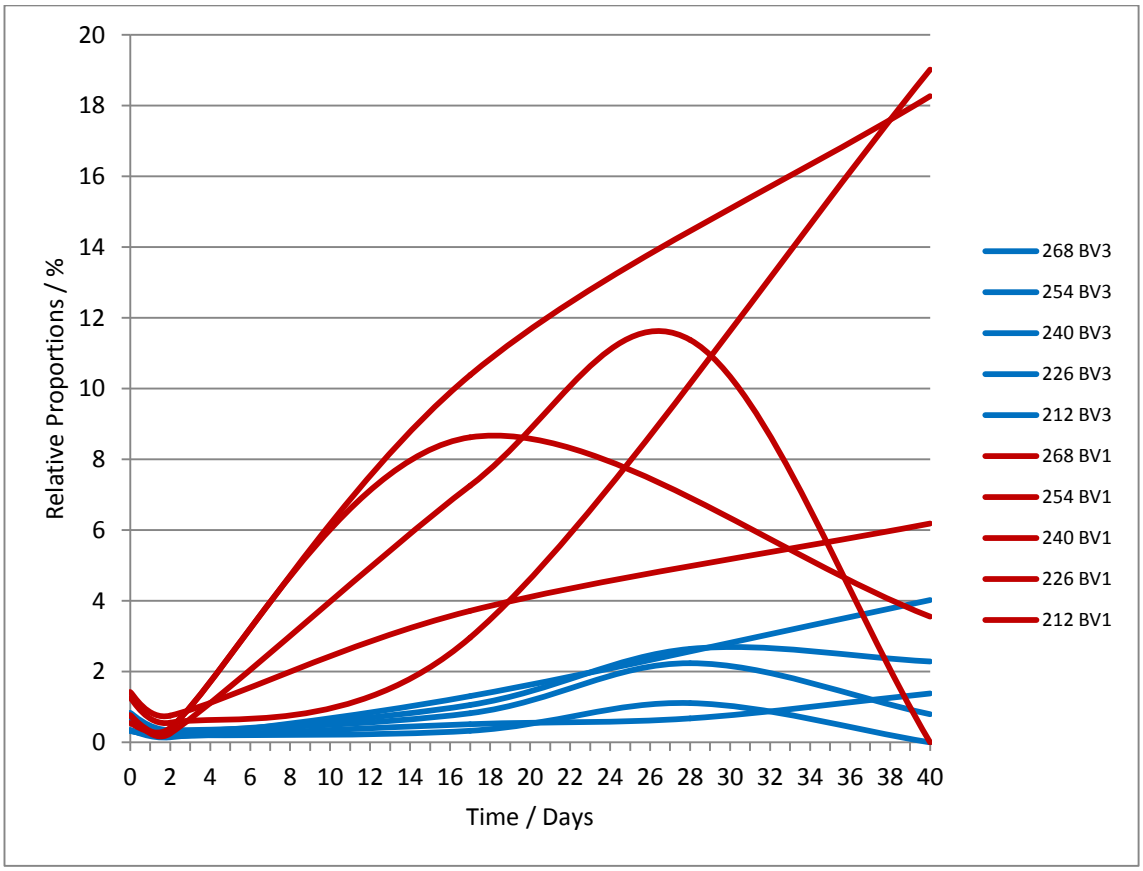


Figure 4.32 Aging Curves for Benzophenone Products for Basic Violet 3 and Basic Violet 1 during Treatment with 5% Bleach

As was seen in the mass spectra for Basic Blue 26, little degradation occurred over the 40 days. This was corroborated in the aging curve of Figure 4.33. As a fresh sample m/z 470 had a relative proportion of 97% and the other components were barely detectable with a total RP below 1%. There was a very gradual decrease of m/z 470 over the 40 days to end with an RP of 87% which was an actual loss of 11%. There was a steady increase in the relative proportion of m/z 456, from 1% to 10% over the 40 days, but no detectable change in the other components. Bleach did not decolourise the Basic Blue 26 dye solution in 40 days.

Relative ion count data also supports that little degradation occurred. At T = 0 m/z 470 was 100% and m/z 456 was 1%. At T = 40 days, m/z 470 was 90% and m/z 456 was 10%.

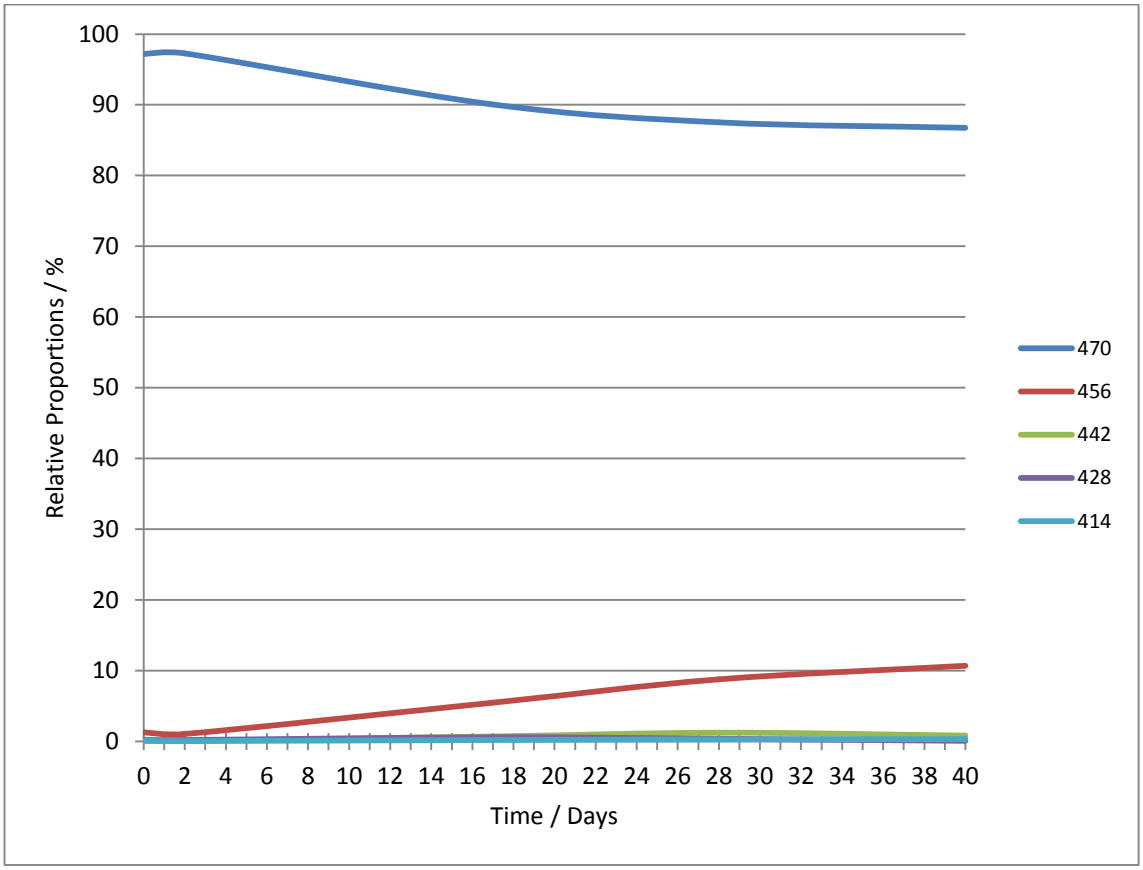


Figure 4.33 Aging Curves for Basic Blue 26 during Treatment with 5% Bleach

Data obtained for benzophenone products from the degradation of Basic Blue 26 after treatment with 5% bleach was not substantial. The relative ion count values detected for all products were below 1% so could not be accurately included. This would corroborate the degradation curves of the primary products as Basic Blue 26 did not degrade significantly over the 40 days and m/z 470 still had a high RP, so benzophenone products have not been produced at this stage. This information shows that for Basic Blue 26, the response of the dye to bleach was different to its response to hydrogen peroxide and it was more stable in 5% bleach than 5% hydrogen peroxide.

Phenol products can also be produced due to the breaking of the central bond. No phenol products were detected for Basic Blue 26. This was not surprising as the degradation of the dye components was minimal and no benzophenones had been produced. For dyes Basic Violet 1 and 3 the RP values were not particularly high, but relative ion count data supports that m/z 137 increased from 0 to 4% for Basic Violet 3 and 0 to 12% for Basic Violet 1.

Table 4.11 shows the relative proportions of the component types when fresh and after 40 days. It shows a reduction in dye components for Basic Violet 3 and 1, with the degradation greater for Basic Violet 1 as it reduced from 94% to 51%, but Basic Violet 3 only reduced by 10%. Basic Blue 26 showed little degradation which was supported by the ion count data indicating a degradation of only 1%. There was an increase in the benzophenone degradation products, but it was significant for Basic Violet 1 increasing from 5 to 47%. This corroborates the results of increased degradation for Basic Violet 1. Phenol products did increase for Basic Violet 3 and 1, but only by 2%. There were no phenol products detected for Basic Blue 26.

Table 4.11 Table to show the Relative Proportions of Products based on Ion Count Data after Treatment with 5% Bleach

	Basic Violet 3		**Basic Violet 1**		**Basic Blue 26**	
	T=0	**T=40**	**T=0**	**T=40**	**T=0**	**T=40**
Dye /%	98	89	94	51	100	99
Benzophenones /%	2	8.5	5	47	0	1
Phenols /%	0	2.5	0	2	0	0

These results have shown that even a small amount of bleach can have an effect on the dyes but the effects are not always the same for all of the dyes.

4.4.1.2 In the Light

In addition to the use of household bleach to treat the dye standards, they were also subjected to artificial daylight in combination with bleach to see what affect it would have on the dyes and the degradation process. The mass spectra for the blue dyes can be seen in Figures 4.34-4.36.

Figure 4.34 clearly shows how the main peaks at m/z 372 and 358 became less abundant and peaks m/z 316 and 288 increased over time. When light was used in combination with bleach, Basic Violet 3 degraded faster than without light. After 5 days, the peaks corresponding to m/z 372 and 358 had significantly reduced in intensity and the known degradation products were being seen. Peaks at m/z 330, 316 and 302 represent the tri, di and mono-methylated homologues for Basic Violet 3 with a mass difference of 14. At 9 days, the final homologue (hexa-

demethylated) m/z 288 was the predominant peak present in the spectra, with all methyl groups lost from the original structure. The other homologues were present but in much smaller quantities.

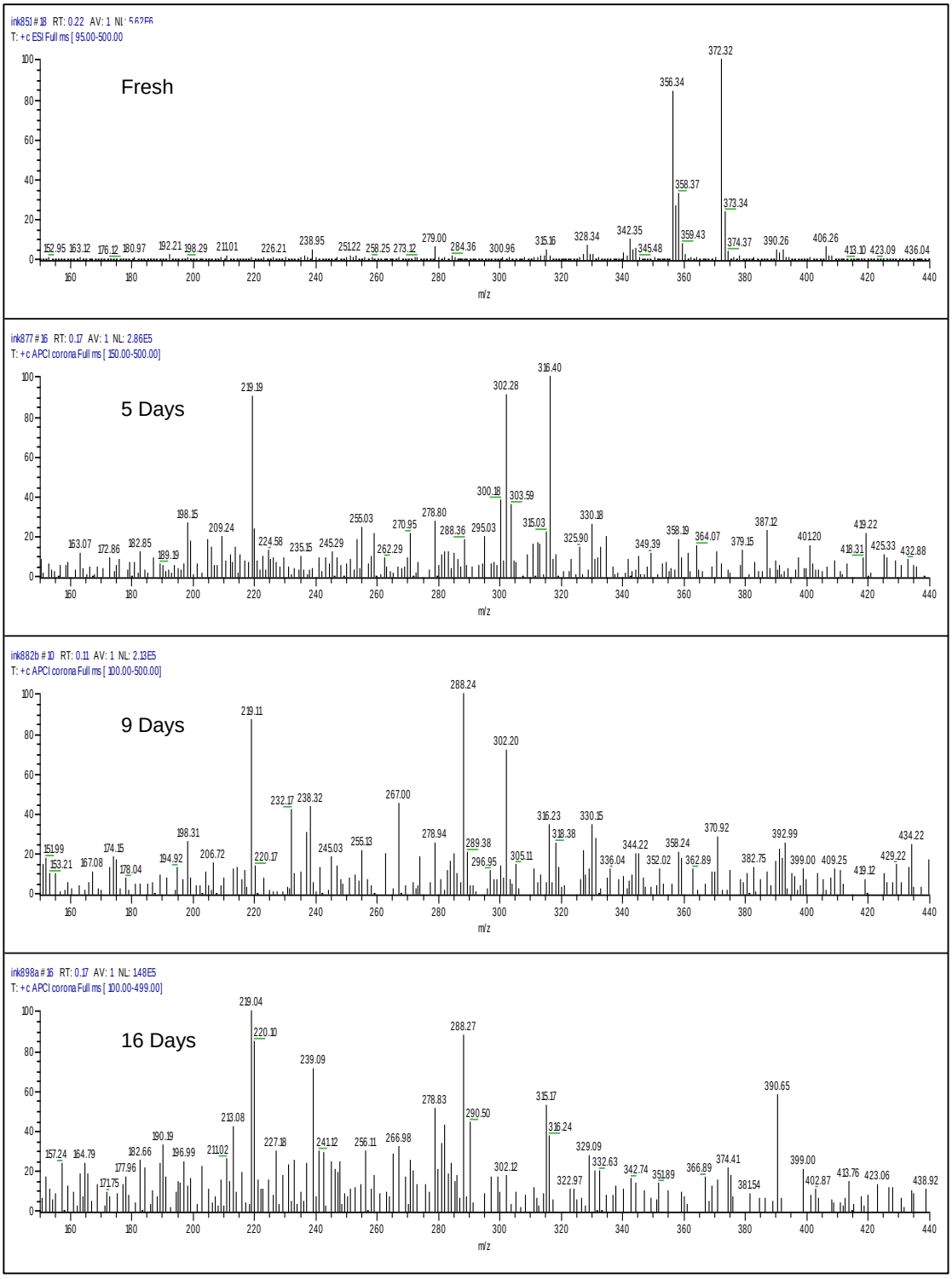


Figure 4.34 APCI-MS Spectra for Basic Violet 3 during Treatment with 5% Bleach and Light

Basic Violet 1 responded to the bleach and light in a similar way that Basic Violet 3 did as shown in Figure 4.35. After 5 days, the predominant peak was m/z 330, (di-demethylated) with 358 and 372 considerably minor in comparison. Peaks at m/z 316 and 302 were present, but unlike Basic Violet 3 m/z 288 was not the main degradation homologue.

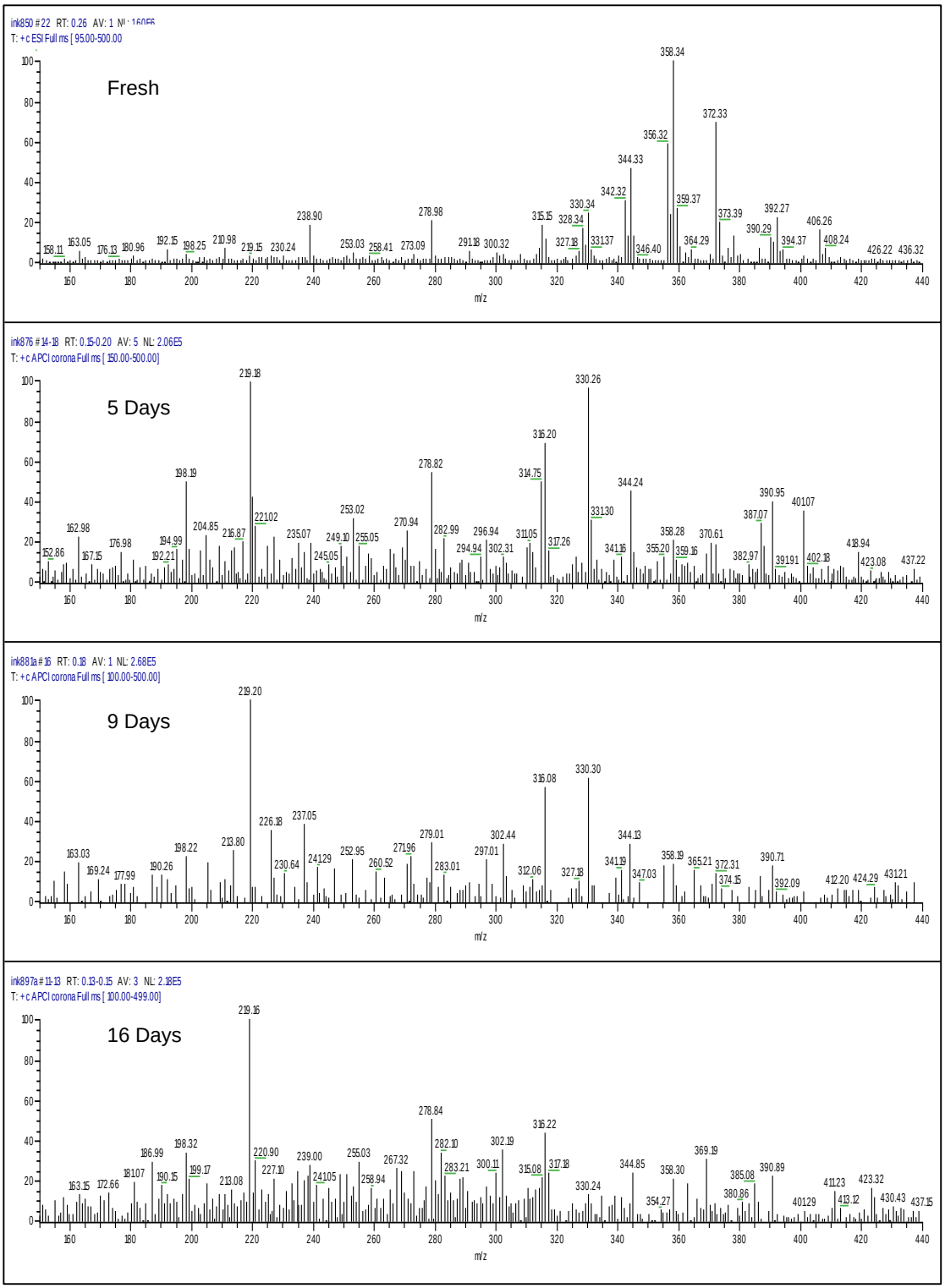


Figure 4.35 APCI-MS Spectra for Basic Violet 1 during Treatment with 5% Bleach and Light

Figure 4.36 shows the degradation of Basic Blue 26 with 5% bleach and light. M/z 470 remained the predominant peak past 9 days, but degradation did occur and was shown by peaks at m/z 456 from 5 days corresponding to the loss of 1 methyl group (–CH_3) and 428 at 16 days; a loss of 3 methyl groups. Basic Blue 26 can potentially lose 4 methyl groups from its structure.

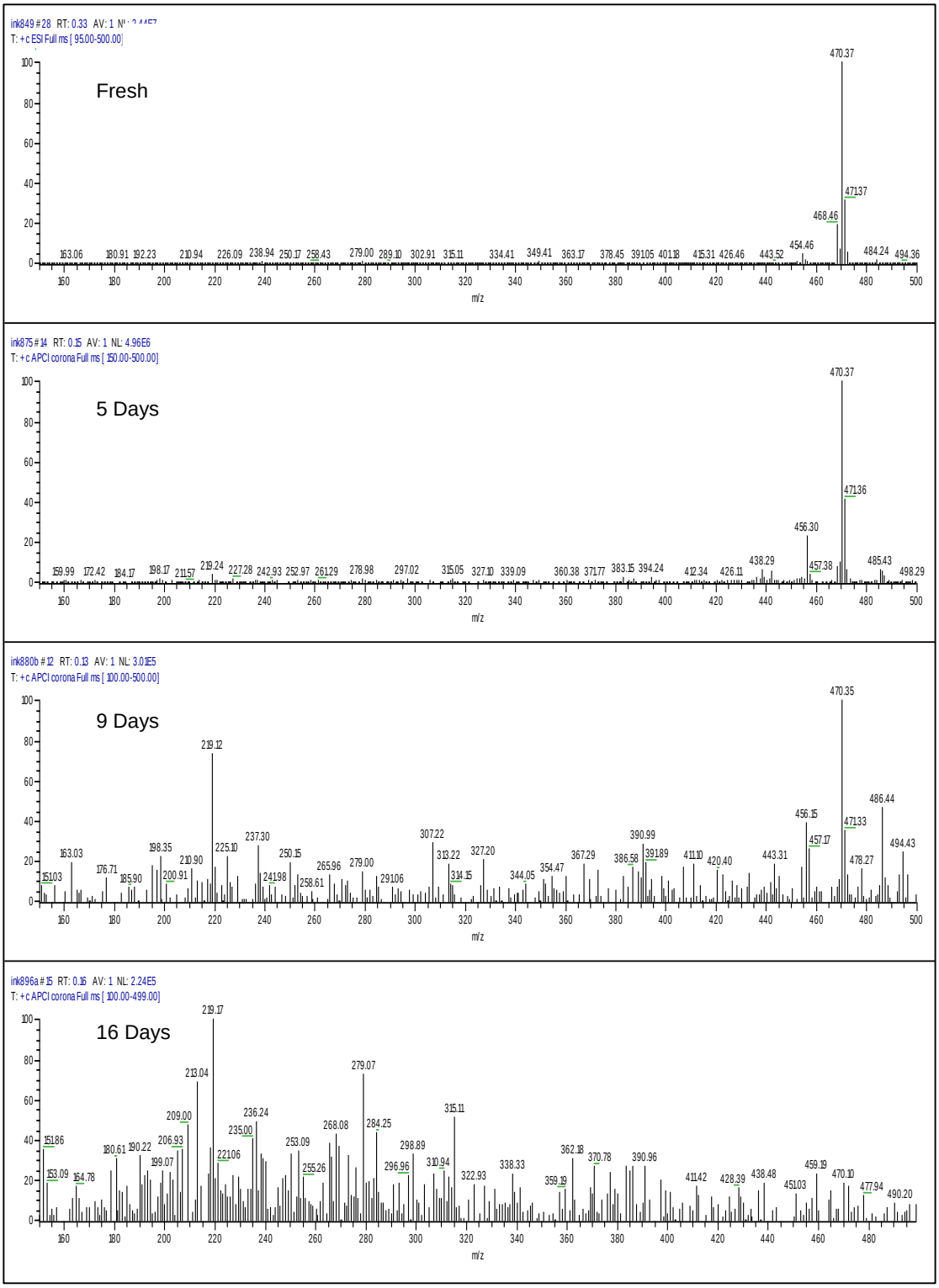


Figure 4.36 APCI-MS Spectra for Basic Blue 26 during Treatment with 5% Bleach and Light

Figure 4.37 displays the aging curves for Basic Violet 3 and 1 following treatment with bleach and light.

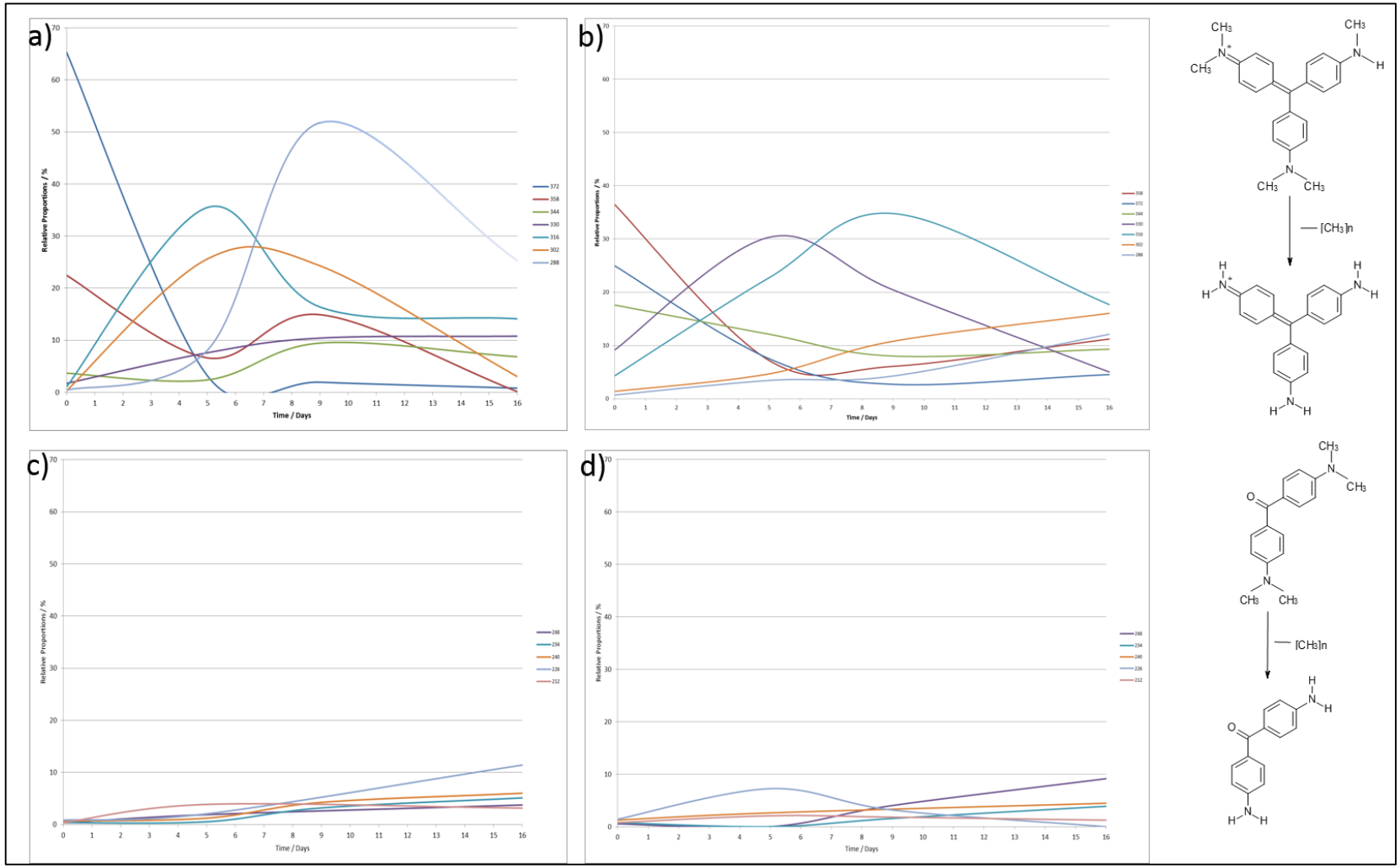


Figure 4.37 Aging Curves for dyye standards a: Basic Violet 3, b: Basic Violet 1, c: Benzophenones for BV3, d: Benzophenones for BV1 during treatment with 5% bleach and light

With bleach and light there was a lot of activity with Basic Violet 3 compared to without light as shown in graph a) in Figure 4.37. All degradation homologues showed an increase in their relative proportions almost immediately which could correspond to the quick degradation of m/z 372 and the production of its products via demethylation. Relative ion count data supports the RP data. At T = 0, m/z 372 was 100% and dropped to 1% by T = 16 days. The most abundant component at 16 days was m/z 288 (0 Me) with a relative ion count of 37%. In comparison, the homologues for m/z 316 (2 Me) and m/z 330 (3 Me) were most prominent with Basic Violet 1. The relative ion data showed that m/z 358 decreased from 100% at T = 0 to 24% at T = 16 days and the most abundant component at 16 days was m/z 316 (tri-demethylated) which had increased from 12% – 38%. This data was different to all other data so far with regards to the degradation of these two dyes. Under all other conditions, Basic Violet 1 appears to go through more degradation than Basic Violet 3, whereas this condition of bleach and light would indicate more degradation for Basic Violet 3.

The aging curves for Basic Blue 26 with light are shown in Figure 4.38. M/z 470 gradually declined over the 16 days and the RP decreased from 97% to 5%. Relative ion count data supports the RP data, m/z 470 decreased from 100% at T = 0 to 13% at T = 16. This was increased degradation compared to bleach and no light. Without light benzophenone products were not detected for Basic Blue 26. This was also the case with light. Even though degradation had increased with light, it had not proceeded to break the central carbon bond.

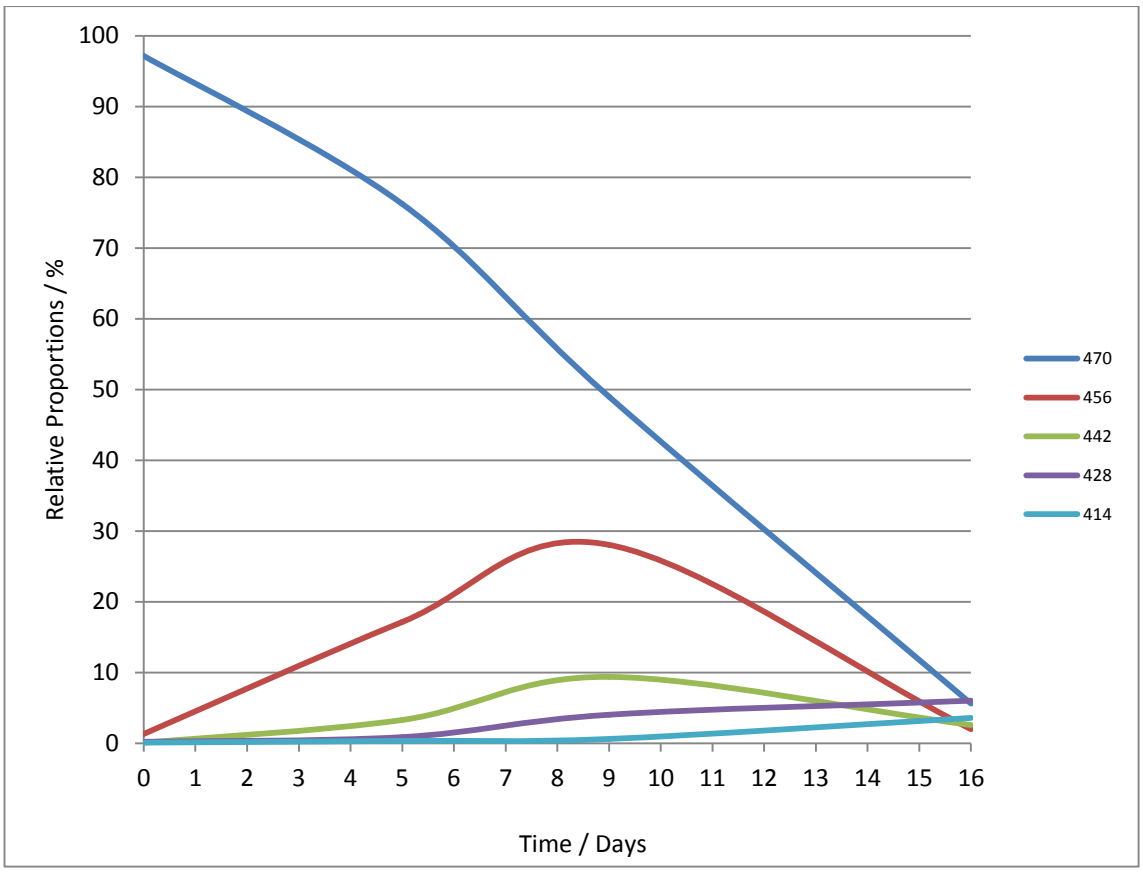


Figure 4.38 Aging Curves for dye standard a: Basic Blue 26, b: Benzophenones for BB26 during treatment with 5% bleach and light

Table 4.12 shows the relative proportions of the component types when fresh and after 16 days based on ion count data. As seen previously for photofading and peroxide treatment the dye components have reduced in amount and the degradation products have increased. Basic Blue 26 appears to have had the greatest amount of degradation as the dye components for this dye have decreased from 100% to 20% in relative proportion. Phenol products have increased for all dyes, unlike without light, when no products were detected for Basic Blue 26.

Table 4.12 Table to show the Relative Proportions of Products based on Ion Count Data after Treatment with 5% Bleach and Light

	Basic Violet 3		Basic Violet 1		Basic Blue 26	
	T=0	**T=16**	**T=0**	**T=16**	**T=0**	**T=16**
Dye /%	98	65	94	75	100	20
Benzophenones /%	2	29	5	20	0	65
Phenols /%	0	6	0	5	0	15

Figure 4.39 shows the comparable aging curves for the blue dye standards. It can be clearly seen that added light has had an effect on their degradation compared to just bleach treatment whereby the degree of degradation has increased. Also, not only does the rate appear quicker, for Basic Blue 26, the values were greater with light unlike the Basic Violet dyes.

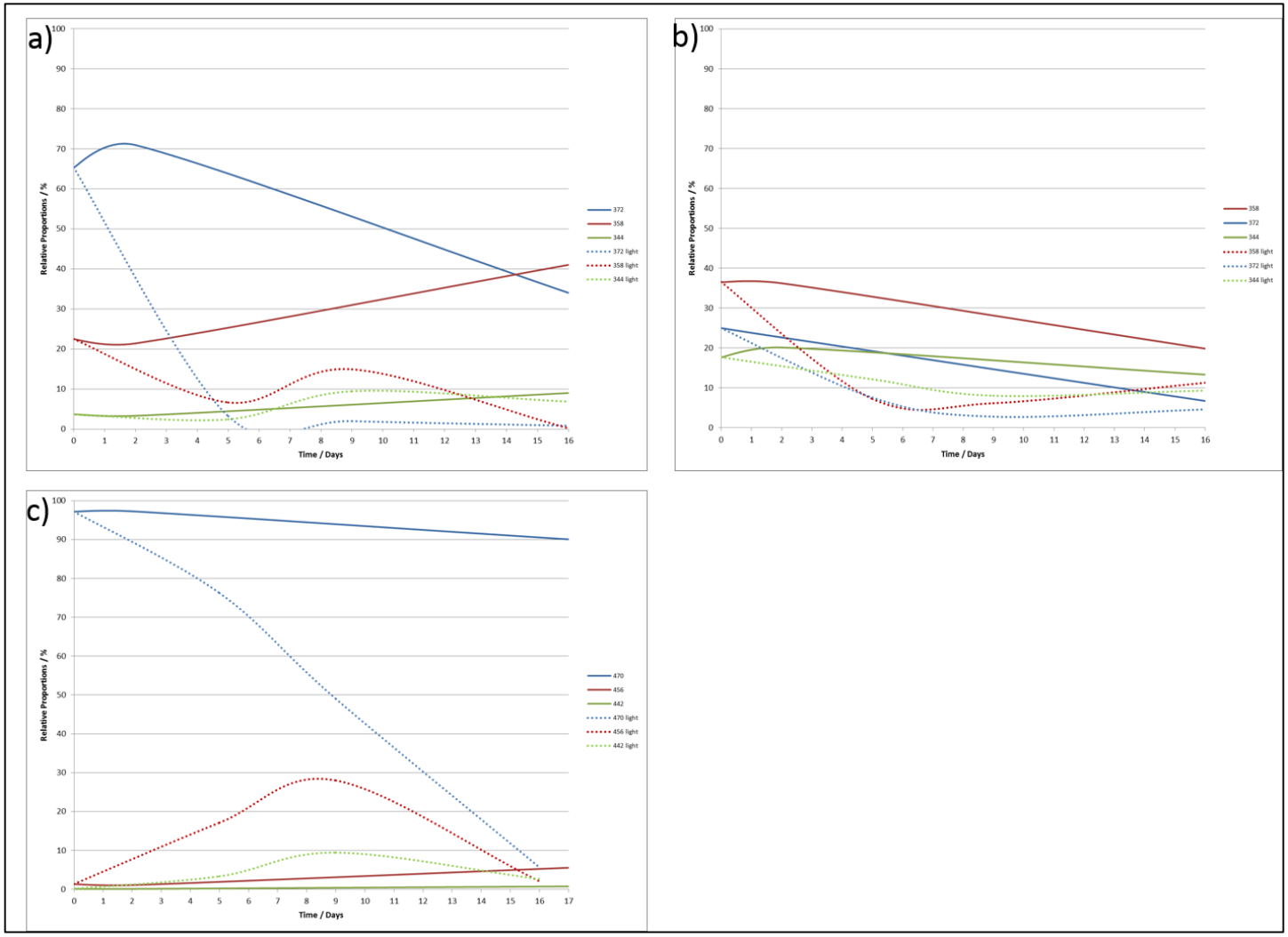


Figure 4.39 Aging Curves dye standards a: Basic Violet 3, b: Basic Violet 1, c: Basic Blue 26 during Treatment with 5% Bleach with and without Light

4.4.2 Bleaching of Inks on Paper

As explained in Chapter 2, bleach was applied to the ink samples when written on the paper surface. Figure 4.40 shows the visible light results after ten applications of household bleach to all samples.

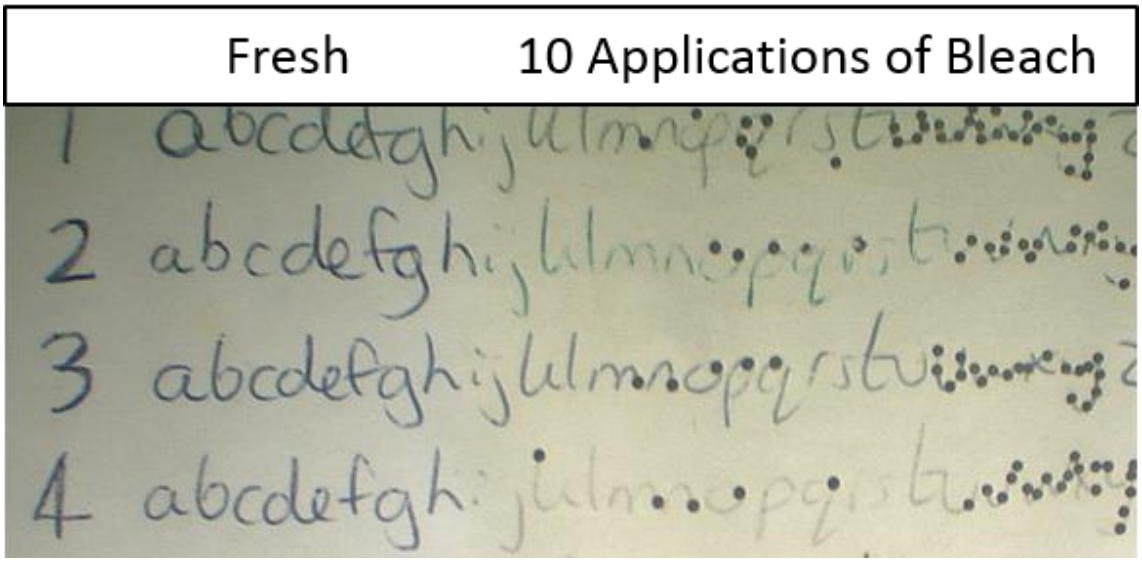


Figure 4.40 VSC Visible Light Image of Fresh Blue Ink and Ink treated with 10 applications of 5% Bleach

After only one application of bleach, the inks were slightly lighter than without, but no significant fading had occurred except for pen 2 –Bic which had lost its blue colour. The other pens have not been affected significantly in terms of their visual appearance. After ten applications fading had occurred for all samples, but not to the same extent as seen in Figure 4.40. Sample 4 (Woolworths) showed the most significant fading, with little colour remaining. Like with the peroxide samples, Bic had changed to a turquoise shade and was the only blue ink to do so. There did not appear to be any affect from the bleach to the immediate vicinity of the ink line like with some of the peroxide samples, but the paper generally did become yellow if subjected to bleach.

Fluorescence was also examined for the bleach samples and the results are shown in Figure 4.41.

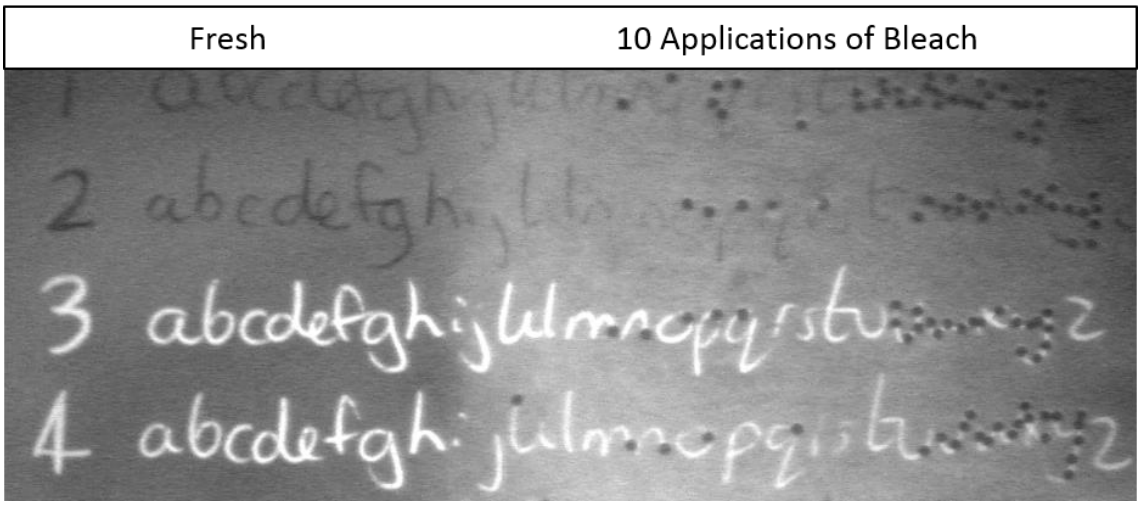


Figure 4.41 VSC Fluorescence of Blue Pen Samples Fresh and after 10 applications of 5% Bleach

Pen samples 3 and 4 both exhibited fluorescence with and without bleach treatment, but for the blue inks no other fluorescence was induced due to bleach treatment. It was also clear to see the area in which the bleach had been applied to the paper (letters j-z) as the paper was brighter.

These results are similar to results from photofading and peroxide bleaching in that fluorescence enabled the re-visualisation of faded ink, but different samples fluoresced under different conditions.

APCI-MS data was analysed to determine the aging curves of the pen inks on paper post bleach treatment as shown in Figure 4.42 for the dye Basic Blue 26 present in pens 1, 3 and 4. Visually ink 3 (Staedtler) was the least faded of the blue samples and ink 4 (Woolworths) was the most faded. The RP values were much lower in ink 4 (graph c) as m/z 470 decreased from 50% to 5% after just one application of bleach. This has led to an increase in the later homologue m/z 414 (0 Me). In graph b this homologue is only just being produced after 3 applications supporting the visual observation of less degradation.

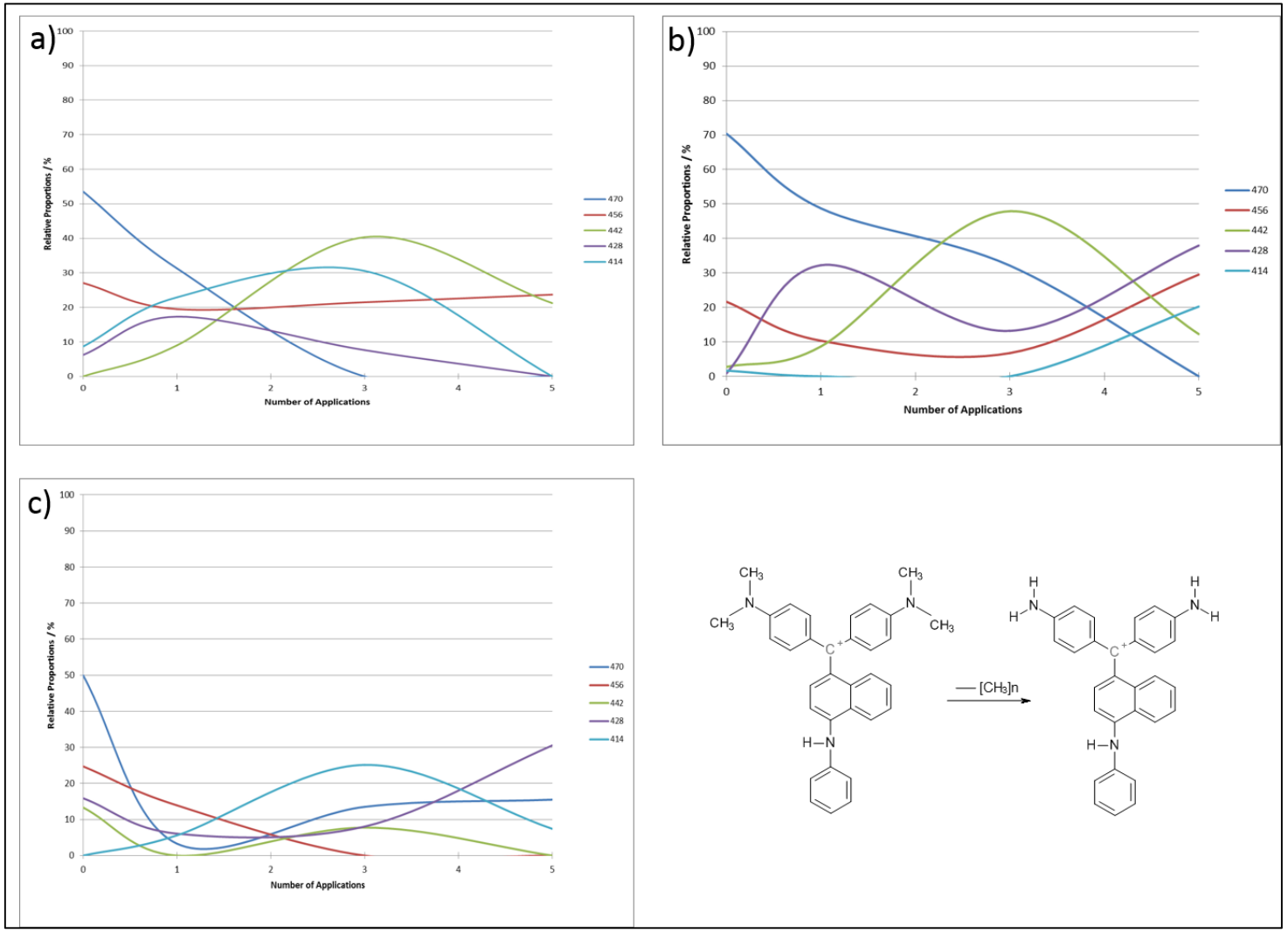


Figure 4.42 Aging Curves for Basic Blue 26 in Blue Pens a: Micron, b: Staedtler, c: Woolworths after treatment with 5% bleach

The aging curves for Basic Violet 3 and 1 in pens 1, 2, 3 and 4 can be in seen in Figure 4.43. For Staedtler there was less activity in the aging curves compared to the other pens, and not until six applications did the degradation homologues increase in proportion. For the other pens, the later homologues m/z 316 (2 Me) and m/z 344 (4 Me) were prominent which was unusual as you would expect some form of successive degradation, but that was not clear here. Relative ion count data for Staedtler showed that m/z 372 went from 85% at T = 0 to 50% at T = 10 applications indicating some degradation, and for Woolworths m/z 372 dropped from 100% to 3% which supports significant fading.

It would appear that with bleach for blue inks, the amount of degradation for Basic Blue 26 was greater than for the Basic Violet dyes, which may account for the fading of the ink colour. There was more Basic Blue 26 in the original composition of ink 3 than ink 4, which may make the dye more stable and less susceptible to attack as demonstrated here, however previously in solution, when more Basic Blue 26 was present, it appeared more susceptible to attack. Discrimination of the inks was much more difficult following bleach treatment, especially for Basic Blue 26, compared to other conditions.

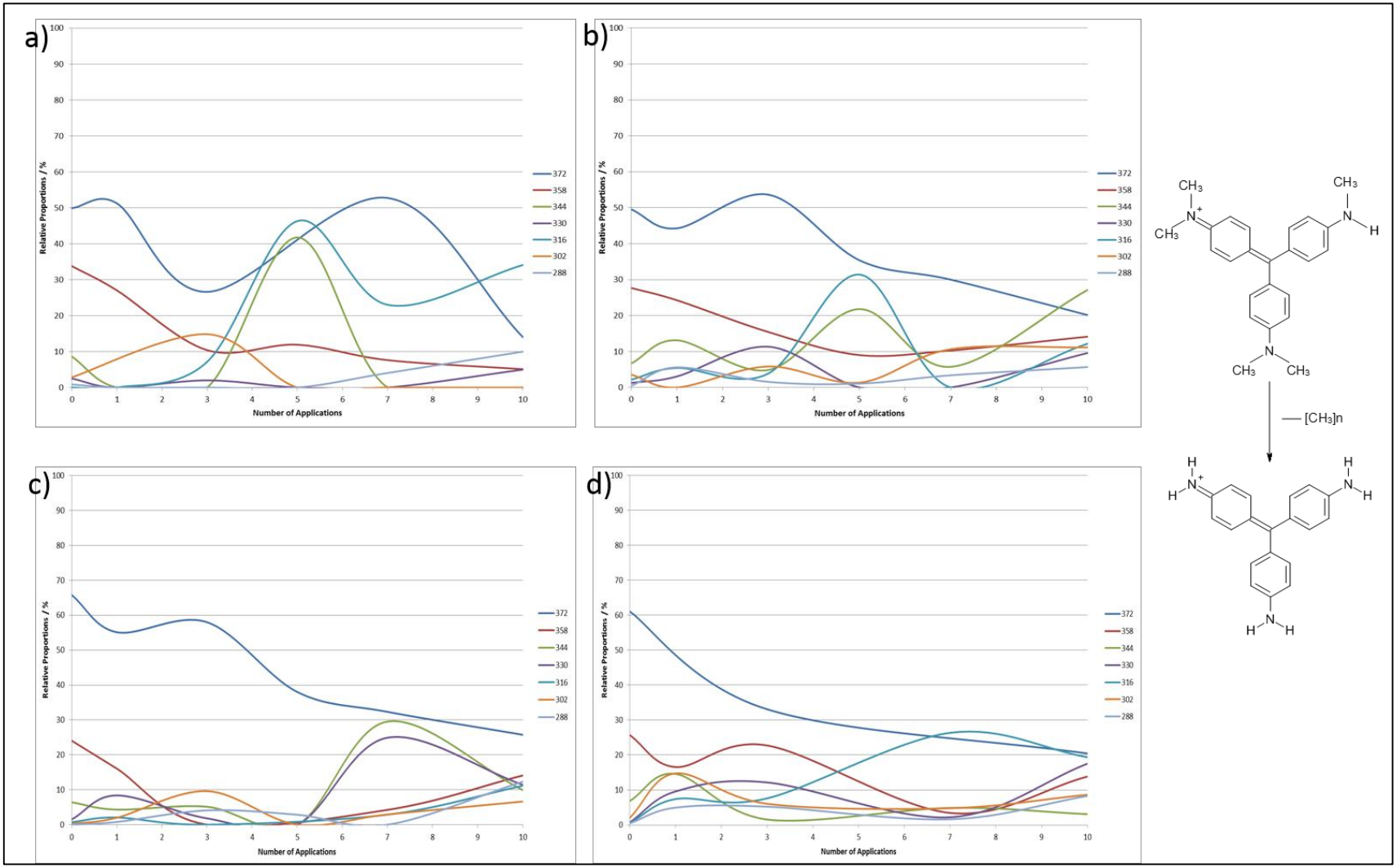


Figure 4.43 Aging Curves for Basic Violet 3 in Blue Pens a: Micron, b: Bic, c: Staedtler, d: Woolworths after treatment with 5% bleach

4.5 Discussion and Conclusions

4.5.1 Photofading

When the samples were exposed to light, the number of degradation products and their quantities increased with time which was consistent with previous research.[18] Furthermore the aging curves clearly confirmed a successive production of the products, which was also consistent with the literature.[154] Their RP values would increase and decrease at different times in their order of degradation, e.g. for Basic Violet 3 m/z 316 reached its peak at 5 days, m/z 302 at 7 days and m/z 288 at 9 days. Basic Violet 1 also showed the succession, but at 5 days m/z 330 was greatest and at 9 days m/z 316. Degradation was clearly enhanced with light, but the rates for all dyes were still different. The degradation of Basic Blue 26 was also enhanced by light but not to the same extent as the other dyes as it was a very stable dye under normal conditions. M/z 470 decreased quite significantly, but the primary degradation products from demethylation did not increase to the same extent and some were still barely detected. However, benzophenone products were detected from 10 days, after which all continued to increase steadily. Relative Proportion and aging curves enable us to see the stability of a dye and its components and how they change over time, but they cannot tell us the rate of degradation. Relative ion count data however, can give information about the degree of degradation and in support of the results from RP, it showed that Basic Violet 1 had a greater degree of degradation that Basic Violet 3.

For the dye mixtures, based only on the parent dye degradation, mixture 1 appeared to exhibit the greatest degradation, however when all the data was considered including benzophenone production, mixture 2 had the greatest degradation. Mixture 2 corresponded to ink 1 (Micron) which visually exhibited little degradation. Mixture 3 corresponded to ink 4 (Woolworths). This ink was the most degraded of the blue samples, but this was not represented in the data for the mixture. These results show that the dyes behave differently when in solution and when on a substrate and investigators need to take care when interpreting case samples on a substrate, and comparing the data to dye standards in solution.

The ballpoint ink samples showed the sequential loss of methyl and ethyl groups, but also the successive production and degradation of products, shown as fluctuating peaks in the aging curves. This however was only present when subjected to external factors such as variable light flux. Dyes degrade strongly under the influence of light, but very slowly or not at all in the dark. Samples stored in the dark in a folder, did not exhibit any significant signs of degradation over the same time period which was consistent with previous studies.[18] If degradation does occur in the dark, further research is required to confirm if the pathways of degradation are via *N*-demethylation or hydration.

Degradation products increased with time exposed to light, but they did not increase continually. They appear to level off after longer exposure times, implying that the degradation process reaches a limit or is slowing down significantly. This can be explained because as a dye degrades via demethylation the wavelengths at which it will absorb are shifting to shorter wavelengths (hypsochromic), for example λ_{max} for Basic Violet 3 that has six methyl groups is 590 nm, but for pararosaniline the homologue that has no methyl groups, λ_{max} is 544 nm. Figure 4.44 shows the light intensity vs wavelength for the solar spectrum which was replicated by the artificial daylight bulbs.

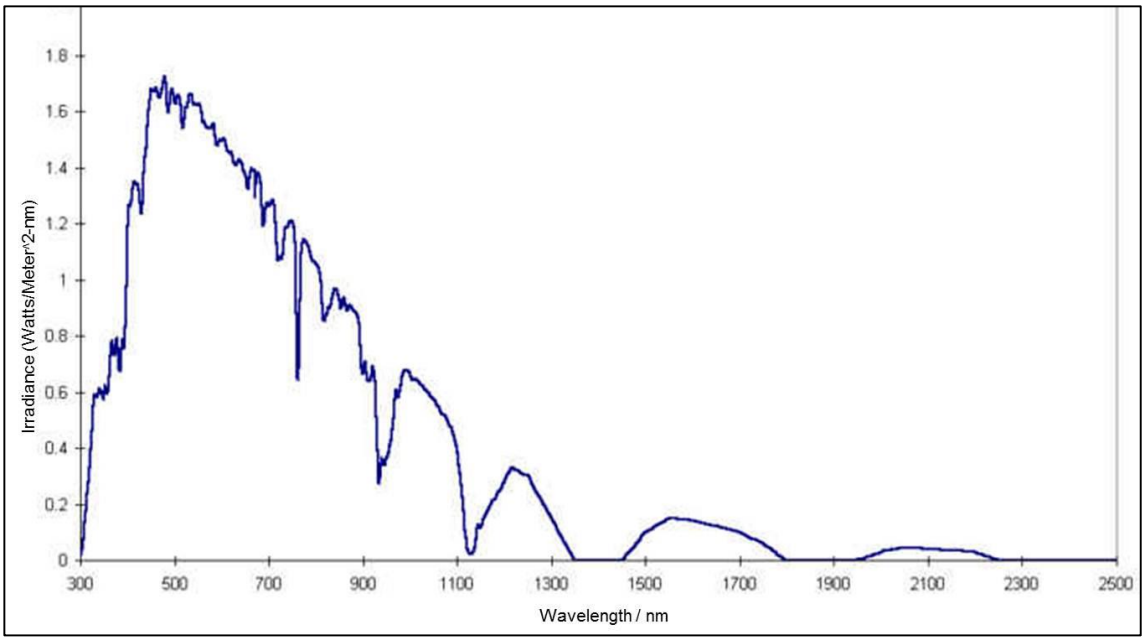


Figure 4.44 Typcial Solar Spectrum

It can be seen that as the wavelength of light decreases, the intensity increases till approximately 500 nm at which point there is a sudden decrease to 300 nm.

As the dyes degrade through the wavelengths and the λ_{max} decreases, the molar absorbance decreases so less light will be absorbed by the molecules, giving lower yields of singlet oxygen and therefore lower yields of degradation products. Also, the width of a typical absorption band peak extends approximately +50 nm to -150 nm from the λ_{max}, so the majority of the absorption band for demethylated dyes will fall progressively further into the <500 nm drop off region, which means less reaction with progressive demethylation. The benzophenone products do fall into the low intensity <500 nm region and so should be stable to daylight, but not to dye generated singlet oxygen, so if there are still dye components present the benzophenones will demethylate.

From analysis of all APCI mass spectra data for photofading it is believed that benzophenone products were present in the ink samples tested and also phenols, however they were present in small quantities.

Results so far support the literature in that light influences the degradation process, but in real situations photofading could occur naturally from sunlight, or deliberately using artificial light sources. New findings concern the use of luminescence to enhance faded entries caused by photofading. Fluorescence was observed in samples after photofading that were colourless, which made the writing visible. Further investigation is needed to determine the exact component causing the fluorescence in the inks.

Other oxidation processes may also be used to affect the fading process; for example hydrogen peroxide can be obtained quite easily and is present in some household products which could be used to alter information written on documents deliberately.

4.5.2 Bleaching with Hydrogen Peroxide

When the samples were treated with hydrogen peroxide, the number of degradation products increased. With 2 µl of 5% H_2O_2, all dye standards had visibly faded and Basic Violet 3, Basic Violet 1 and Basic Blue 26 were colourless by 8 days. The successive degradation and subsequent production of

homologues seen with photofading were not apparent when peroxide was used and there does not appear to be an order in which the products were formed. Degradation was occurring however from peroxide and the lower homologues e.g. m/z 302 and 288 for Basic Violet 3 and 1 and m/z 414 for Basic Blue 26 were the predominant products at the end of the analyses. For Basic Blue 26, m/z 470 decreased, but the typical products were not produced in significant quantities which would suggest that the degradation pathway for Basic Blue 26 with peroxide was different than with light. Benzophenone products were also present with m/z 254 and 226 prominent for all three dyes and the RP values between 15 – 35%. Phenol compounds were detected in differing amounts for the different dyes for example Basic Violet 3 had values of 6%, but for Basic Violet 1 all values were below 2%. There is limited information about reactions between hydrogen peroxide and dyes in the absence of light. Some studies have said that decolourisation does not occur when only H_2O_2 is used,[157] another stated that a small amount of colour loss does occur by H_2O_2 alone.[153] These papers were interested in dyes for environmental reasons and the conditions of the research were different to those used in this thesis, for example, different dyes and different techniques used to analyse the photochemical oxidation of the dyes, so the exact results will not be the same.

When light was used in combination with peroxide, degradation occurred as would be expected, and it appeared that light increased the degradation. Once again, for Basic Blue 26, there were no significant degradation homologues produced, and for all dyes a lack of successive degradation see by photofading alone. Benzophenone and phenol products were again produced, but with different values to when only peroxide was used; for example, for Basic Blue 26, the RP values were higher at 6%.

As was seen with the mixtures during photofading, some synergistic effects were observed with peroxide bleaching as well. The degree of degradation of Basic Violet 3 and 1 would be increased if there was more Basic Blue 26 present in the mixture. It is known that dyes will compete for reactants, such as light photons and in the process they quench the degradation of the other dyes.[150] A difference was observed between photofading and bleaching for the mixtures. In light, the later homologues (m/z 288, 316) would be present and have the highest RP

values in the aging curves, but in peroxide bleaching, the early homologues (m/z 344, 330), would be present with the highest values. This would indicate that the degradation process was occurring in a different way under the two conditions; in light, the process may be successive and the homologues were generally formed one after the other, whereas in peroxide, the degradation could be quicker and the homologues were not produced at a rate that was quicker than they are destroyed, which would actually produce more of the later homologues. Benzophenone products were detected and their RP values were largely higher from peroxide bleaching than photofading. This could be explained because hydroxyl radicals (which can be formed from hydrogen peroxide) have been recorded as attacking the chromophore structure leading to the generation of benzophenone and phenol products.[158] Also when light and peroxide are used in combination, singlet oxygen would be produced by some dyes due to the light and hydroxyl radicals produced by the peroxide which would mean more reactions would be happening and consequently the degree of degradation would be faster. The degradation that appeared to be the greatest, according to the ion count data, occurred for mixture 1 which corresponds to ink 2 (Bic). As seen with photofading, these results did not match the visual degradation or the aging curve data of the Bic ink. These results again show that the dyes behave differently when in solution and when on a substrate.

On paper, visible results were seen from peroxide bleaching of the ink, but the fading of the inks was not uniform, showing that the rates of degradation for different inks and therefore different dyes was not the same. It also highlighted how some inks were more resistant to attack than others. The fluorescence was useful however for a number of samples that fluoresced before and after treatment and were not legible, as it allowed the text to be seen. This is a novel way of using luminescence that has not been documented previously.

When more hydrogen peroxide was applied to the ink on paper, it was expected that degradation of samples would increase and therefore the amount of products would increase. In some samples this was true, however, as the results for the benzophenone products showed, they mostly peaked after nine applications of peroxide and their values decreased after this. An increase in H_2O_2 will lead to faster degradation of samples but only to a certain level. It has been recorded

previously that there is a critical level at which H_2O_2 will be effective and if the concentration is too high, degradation actually becomes slower. This is due to "self-quenching" of the H_2O_2 as it competes for the hydroxyl radicals.[153,160]

Hydrogen peroxide has shown to increase the rate of degradation of the dyes and the degradation products; benzophenones and phenols have been detected, but it also appears to have a negative effect on the products. Hydrogen peroxide is a powerful oxidant and it was actually destroying any molecules that resulted from the degradation of the dyes as well as the dye compounds, which would explain why the products' values were low and sometimes were not present for very long, with large fluctuations in value over time.

The ballpoint ink samples showed signs of demethylation and deethylation, but not necessarily in the homologue order that would be expected. As was seen, benzophenone products were evident following the degradation of the dyes, as were phenol products, which were due to the breakage of the central phenyl bond. They were not produced however sequentially as demethylation or deethylation but concurrently. In some cases these products were detected almost immediately following treatment. Hydrogen peroxide produces hydroxyl radicals and according to the literature, the primary attack by hydroxyl radicals is to the chromophore.[158] Research has shown that when TiO_2 was used to photocatalyse Basic Violet 3, there were two competitive processes, N-demethylation and destruction of the conjugated structure.[158] Results from this current study have shown that the processes were occurring simultaneously. Previous research has only been carried out on dye standards and specifically with regards to removing the dyes from wastewater for environmental reasons. No research has been published that has looked at dyes solely for ink purposes. Results to this point have agreed in some parts with the literature that hydrogen peroxide will affect the degradation of inks and dyes.

Hydrogen peroxide is just one bleaching agent that can be used to fade ink, another is domestic bleach which contains sodium hypochlorite.

4.5.3 Bleaching with Sodium Hypochlorite.

When the samples were treated with sodium hypochlorite bleach some visual fading occurred, and some degradation products were detected. With the addition of 5% household bleach that has a sodium hypochlorite content of less than 5%, all standard dyes had some degree of fading within a couple of days, but Basic Violet 1 and 3 had the greatest difference in their colour. The successive degradation and subsequent production of homologues seen with photofading was not as apparent when bleach was used. Homologues were produced but there did not appear to be any particular order for their production. Degradation was occurring as different products were detected, but the lower homologues such as m/z 288 and 414 never had large values. The lack of lower homologues shows that with 5% bleach, the degree of degradation was less compared to the addition of peroxide or light. Differences were detected between Basic Violet 1 and Basic Violet 3 that were highlighted in the aging curves. It appeared that the rate of degradation was faster for Basic Violet 1 than for Basic Violet 3, which was consistent with the results from photofading and peroxide bleaching. The bleach also highlighted the difference in the degree of degradation between all dyes. Basic Blue 26 showed little degradation over the time period, and the initial base peaks remained quite stable throughout. Few degradation products were detected, only m/z 456 for Basic Blue 26. Even though the dye standards were initially of a similar concentration, their degree of degradation was quite different.

In addition to demethylation and deethylation, the central bond can be broken as part of the degradation process to form benzophenone products. These were detected following bleach treatment for Basic Violet 1 and 3 but not for any other dyes. The relative proportions were much higher for Basic Violet 1 than for Basic Violet 3 indicating the amounts detected were higher, so more has been produced which supports the results that the degree of degradation was greater for Basic Violet 1 than Basic Violet 3. Over the 40 days m/z 254 was the predominant peak for Basic Violet 3, although all products began to increase steadily. M/z 254 continued to increase whereas the other products decreased after 28 days. For Basic Violet 1, m/z 254 was also the dominant product, but m/z 240 continued to increase throughout the time as it was being produced by degradation. This could show that Basic Violet 1 was ahead of Basic Violet 3 in terms of the degradation

process if they were following the successive homologue process. No substantial benzophenone products were detected for Basic Blue 26, which was consistent with the aging curve data obtained. It would not really be expected for benzophenone products to be present if no dye homologues were present. Phenol compounds were detected for the Basic Violet dyes but in differing amounts. The values for Basic Violet 1 at 6% were higher than Basic Violet 3 at 2% which was consistent with an increased degree of degradation. No phenol compounds were detected for any other dyes.

There is no information available which refers to the reactions of inks with bleach, but there is some information about the reactions of some dyes in wastewater with sodium hypochlorite, but the conditions and dyes used for that research were different to those used in this research. It was observed that bleached seawater using sodium hypochlorite can oxidise pollutant dyes and remove the colour from the water.[161]

When light was used in combination with bleach, the degree of degradation was increased for all dyes which was consistent with peroxide bleaching and light. There were increased values for dye homologues and evidence of successive production seen with photofading was observed. There were also increased values for benzophenones and phenol products for all dyes tested. What was different to previous results was the greater degree of degradation for Basic Violet 3 over Basic Violet 1. Previously Basic Violet 1 had always had the greater degradation, but under these conditions, Basic Violet 3 did. Also Basic Blue 26 had the greatest reduction in ion count data than any other dye, which would indicate the greatest degree of degradation which was different to any previous results.

On paper, visible results were seen from hypochlorite bleaching of the ink and the fading of the inks was not uniform, showing that the degree of degradation for different inks and therefore different dyes was not the same, which was consistent with peroxide bleaching. After one application of hypochlorite bleach, blue Bic had faded much more than the other inks. The results of bleaching on paper were similar to the dye standards, in that few homologues were produced. Degradation was occurring as values of base peaks were decreasing after treatment but values for homologues were not increasing, and the main dye component

remained the most abundant component during the analyses. For the blue inks when Basic Blue 26 and Basic Violet 3 were both present, Basic Blue 26 had a greater degree of degradation and none of its dye components (homologues) were detected for any blue inks following the fifth application of bleach. An interesting observation has been the apparent lack of complete degradation of the dyes when observing chemical data, even though visibly the colour has all but disappeared. When samples were no longer blue following bleach treatment, the dyes were still detected in their original form, and there was a distinct lack of homologues for some inks. The degradation process that occurred due to bleach was different to the process that occurs with other chemicals. It is possible that the bleach was reacting with other components of the ink, and not just the dyes. This may explain why the dyes were behaving differently to comparative inks under other conditions. It is also possible that the degradation of the dyes was only partial, and not complete as found by research on wastewater.[162]

The ballpoint ink samples showed signs of demethylation and deethylation, but not necessarily in the homologue order as expected. Benzophenone products were present following the degradation of the dyes which were produced due to the breakage of the central phenyl bond. They were not produced after demethylation or deethylation but at the same time. In some cases they were detected almost immediately following treatment, indicating that the degradation processes were occurring simultaneously.

No phenol products were detected for any of the ink samples on paper following bleach treatment, which was inconsistent with the previous results. However previous research looking at colour removal with sodium hypochlorite of dye wastewater also found that phenols were not produced in the reactions.[162] No research has been found that has documented the effects of sodium hypochlorite bleach on dyes specifically with a focus on ink.

Results have shown that bleaching (whether from sodium hypochlorite or hydrogen peroxide), or photofading will affect the degradation of inks and dyes and in different ways. Degradation products such as benzophenones and phenols have been detected for the different conditions and have proved useful for discrimination purposes.

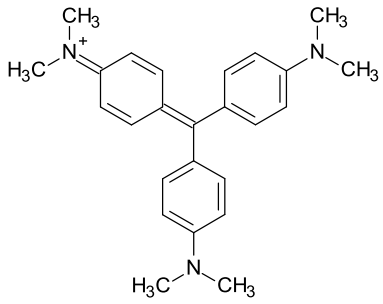
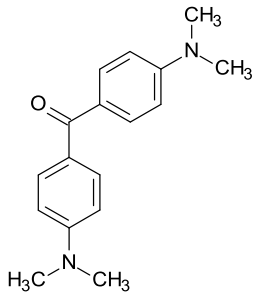
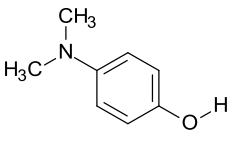
Chapter 5 Analysis of Black Inks

This Chapter will look at the degradation of dye standards, mixtures and inks of black pens when subjected to light, peroxide and bleach.

5.1 Degradation Products

As identified in Chapter 3, the black pens predominantly consist of Basic Violet 3 and Basic Violet 1. Typical degradation products for Basic Violet 3 and Basic Violet 1 are shown in Table 5.1. As structurally both Basic Violet dyes are very similar, their degradation will follow the same pattern. The products in the Table are possible fragments that correspond to m/z as derived from APCI data and relevant literature and are likely to be seen in the APCI spectra for the samples.

Table 5.1 Possible Degradation Products for Basic Violet 3 and Basic Violet 1

Structure	m/z	Compound
	372	$C^+ (Me)_6$ (BV3)
	358	$C^+ (Me)_5H$ (BV1)
	344	$C^+ (Me)_4H_2$
	330	$C^+ (Me)_3 H_3$
	316	$C^+ (Me)_2 H_4$
	302	$C^+ (Me)_1 H_5$
	288	$C^+ H_6$
	268	N,N-Dimethylaminobenzophenone (DDBP$(Me)_4$)
	254	DDBP $(Me)_3$
	240	DDBP $(Me)_2$
	226	DDBP $(Me)_1$
	212	DDBP H_4
	137	N,N-Dimethylaminophenol (DAP)
	123	DAP $(Me)_1$
	109	DAP H_2

The initial reactions that take place are due to *N*-demethylation – the loss of methyl (-CH_3) groups from the nitrogen group. As Basic Violet 3 has six methyl groups attached to the aromatic amine substructures of the cation (C^+), there could be a successive loss of all six of these groups, with multiple isomers occurring for the di-, tri- and tetra-demethylated molecules. Basic Violet 1 has five methyl groups, so can only lose those five. There is a change of 14 atomic mass units as photooxidation converts the N-methyl groups to methanal or methanoic acid, effectively replacing the methyl groups by hydrogen atoms (-H). This is known as oxidative demethylation. [35,66,66,38] Another possible pathway for degradation is via cleavage of the central carbon-aryl bond which leaves a benzophenone product.[35,64] These too may have various numbers of methyl groups attached, so successive loss of these groups can also be seen. The complementary part of the structure forms a phenol as shown in Table 5.1.

5.2 Photodegradation

The dye standards were faded photochemically in solution and ink was faded on paper by the methods described in Chapter 2. The photodegradation products of both the pure dyes and the ink mixtures were analysed by APCI-MS over six days for the standards and 10 days for the mixtures to monitor the aging and fading processes and to determine whether there were any synergistic effects of one dye on the fading of another, e.g. by one dye producing excessive amounts of singlet oxygen which can then attack and increase the fading rate of other dye molecules.

5.2.1 Photofading of Dye Standards in Solution

Each dye standard was faded using an artificial daylight source as described in Chapter 2 to replicate the degradation process of natural photofading. Figure 5.1 shows the aging curves for demethylation for Basic Violet 3 and Basic Violet 1 along with the benzophenone products.

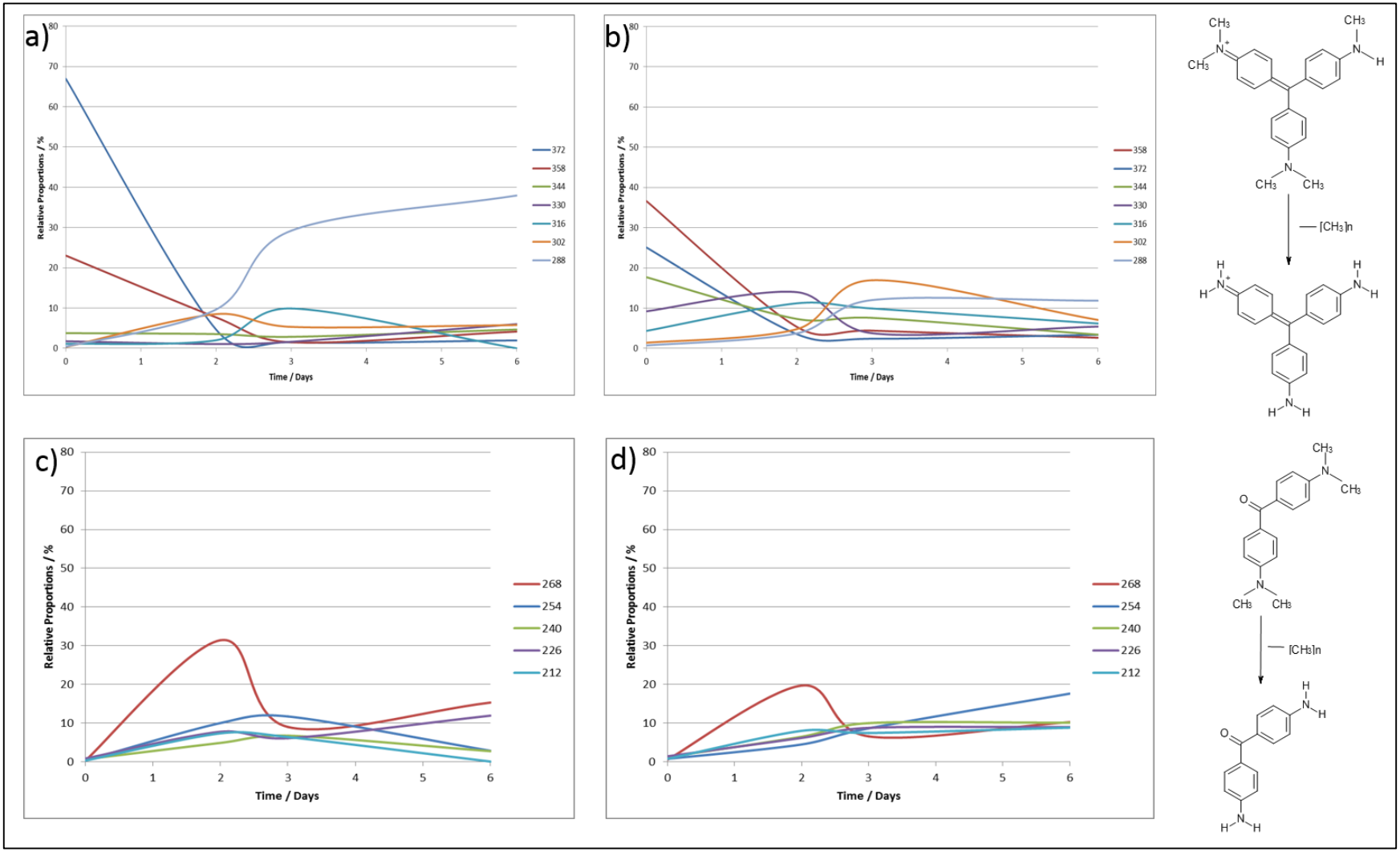


Figure 5.1 Aging Curves for dye standards a: Basic Violet 3 b: Basic Violet 1, c: Benzophenones for BV3 d: Benzophenones for BV1 during Photofading

Graphs a) and b) in Figure 5.1 show that the relative proportions of the main dye components, Basic Violet 3 (6 Me) and Basic Violet 1 (5 Me), decreased significantly over time. This is supported by the relative ion count data which shows that the degree of degradation was quick for both dyes. At T = 0 both dyes were 100% but at T = 6 days Basic Violet 3, m/z 372 was 2.5% and Basic Violet 1 m/z 358 was 7.5%. This means that significant degradation of both dyes took place in just six days for their values to reduce by these amounts, and the degree of degradation was almost the same for both dyes. The relative proportion of the lower homologues 288 (0 Me) showed a significant increase compared to the other products, which would not have been expected until the higher homologues had degraded. This was probably due to a combination of two factors: (i) the hypsochromic shift (to higher energy) of the absorption as the dyes demethylate progressively and (ii) the proportion of the light from the artificial daylight source decreases as it goes to higher energy, resulting in a lower rate of degradation. As the RP values were small for all homologues and of similar value, this indicates that apart from m/z 288, no homologue contributed more to the composition than any other. This was completely different for Basic Violet 1 as all homologues apart from m/z 344 increased immediately and there was a clear succession in their production and peak RP values. The demethylation products m/z 330 (3 Me) and 316 (2 Me) increased initially which would be due to other higher homologues breaking down and producing them, but then they themselves break down explaining the subsequent decrease in their proportions. Relative ion count data for the homologues showed that the values were much higher for Basic Violet 1 than 3. M/z 330, 316 and 288 reached values of 70%, 56%, 37% respectively during the time, but for Basic Violet 3 the same components never exceeded 28%. This would indicate that the degradation of Basic Violet 1 was faster than Basic Violet 3 as the degradation products constituted much higher proportions. These two dyes had not completely faded in six days, but they were colourless by eight days of exposure to light.

Graphs c) and d) in Figure 5.1 show the other degradation products that were produced by cleavage of the central phenyl bond. The most abundant component at two days for both dyes was m/z 268 (4 Me) with a RP of 32% and 20% respectively indicating that it was produced quickly once the parent dye was subjected to light. This would be the first benzophenone expected as it has 4 CH_3

groups still attached. The higher value for m/z 260 in Basic Violet 3 could correspond to the significant drop in m/z 372 over the first 2 days seen in graph a), which is not as dramatic for Basic violet 1 in graph b). The other products also increased in proportion but increased for the six days for Basic Violet 1. M/z 254, 240 and 212 for Basic Violet 3 decreased in RP after three days. All products were still being produced in slightly higher proportions for Basic Violet 1 rather than 3. This can be seen clearly in Figure 5.2. This is corroborated by the relative ion count data, as all values were higher for Basic Violet 1 supporting that degradation was faster for this dye. At T = 6 days, values for Basic Violet 1 ranged between 25% – 50%, but were only 1% – 20% for Basic Violet 3.

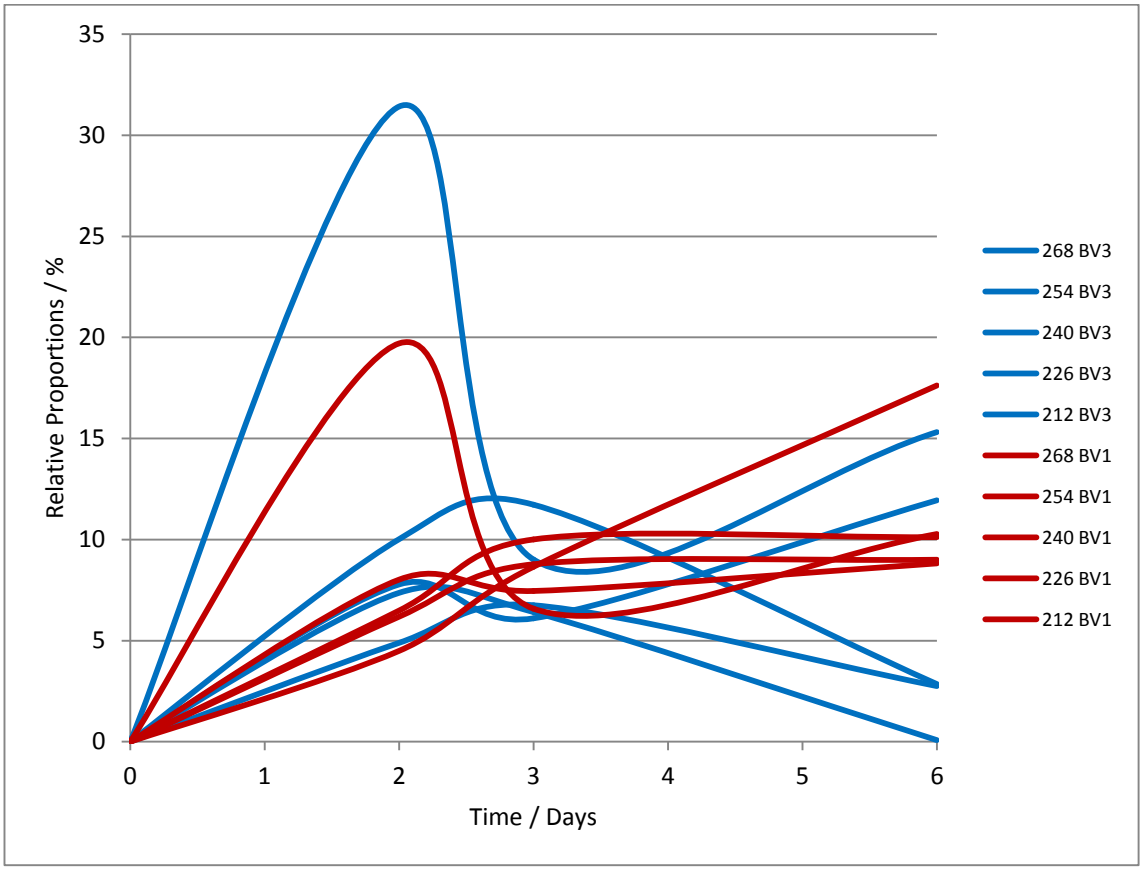


Figure 5.2 Aging Curves for Benzophenone Products from Basic Violet 3 and Basic Violet 1 during Photofading

Phenol products were not detected in high enough quantities to confirm their presence for either of the dyes.

Table 5.2 shows the relative proportions of the component types when fresh and after six days.

Table 5.2 Table to show the Relative Proportions of Products based on Ion Count Data after Photofading

	Basic Violet 3		Basic Violet 1	
	T=0	**T=6**	**T=0**	**T=6**
Dye /%	97	60	95	40
Benzophenones /%	3	34	5	55
Phenols /%	0	6	0	5

The data shows a reduction in all of the dye components, and a greater reduction for Basic Violet 1 than Basic Violet 3. Basic Violet 1 reduced from 95% to 40%, and Basic Violet 3 was 97% to 60%. The benzophenone products increased for all dyes, with once again a greater increase for Basic Violet 1, however, the phenol increase was almost the same for both violet dyes at 5% and 6%. This information would suggest that benzophenone and phenol products were present.

5.2.2 Photofading of Dye Mixtures in Solution

By using dye combinations identified from the ink compositions of the blue ballpoint pen samples shown in Table 3.1, 5 black dye standard combinations were produced as displayed in Table 5.3 for analysis to replicate ink degradation by light. APCI-MS analysis was carried out over 10 days and aging curves produced of the results.

Table 5.3 Dye Standard Dyes

Dye Standard Combination	**Dye standards proportions**	**Pen Colour**	**Pen number**
4	BV1 100 : BV3 40	Black	5
5	BV3 100 : BV1 40	Black	6
6	BV1 100 : BV3 65	Black	7
7	BV1 100 : BV3 85 : BR1 50	Black	8
9	BV3 100 : BV1 100	Black	15

Figure 5.3 shows the aging curves from demethylation for the black mixtures. Generally the components of the dyes behaved in a similar way irrespective of the ratio. In all mixtures, the later homologues, m/z 316 and 288 increased in relative proportion before any other components and their proportions were higher. There was no evidence of successive degradation of the homologues.
M/z 316 was prominent in all mixtures, this could be due to the probability of producing this structure for both dyes. M/z 316 has two methyl groups, they can either be on the same ring or on different rings in the molecular structure, and both dyes are capable of producing it, which may be why the relative proportion for this particular component was higher. The total ion count data provided evidence that the degree of degradation was greater for Basic Violet 1 than Basic Violet 3. In all mixtures the count dropped from 100% at T = 0 to less than 10% at T = 10. M/z 372 also dropped to less than 10% but the starting values were half those of m/z 358 which would indicate that the degree of degradation for Basic Violet 1 was double that of Basic Violet 3.

Mixture 7 contained 3 dyes, Basic Violet 1, Basic Violet 3 and Basic Red 1 in a ratio of 8.5:10:5. The RP values for the Basic Violet dyes in this mixture were much lower than in the other black mixtures. This could be due to the influence of Basic Red 1, similarly to how Basic Blue 26 affected the Basic Violet dyes in the blue mixtures. This information highlights the instability of the Basic Violet dyes and how the proportions of the dye components can change significantly over a short period of time and when other dyes are present.

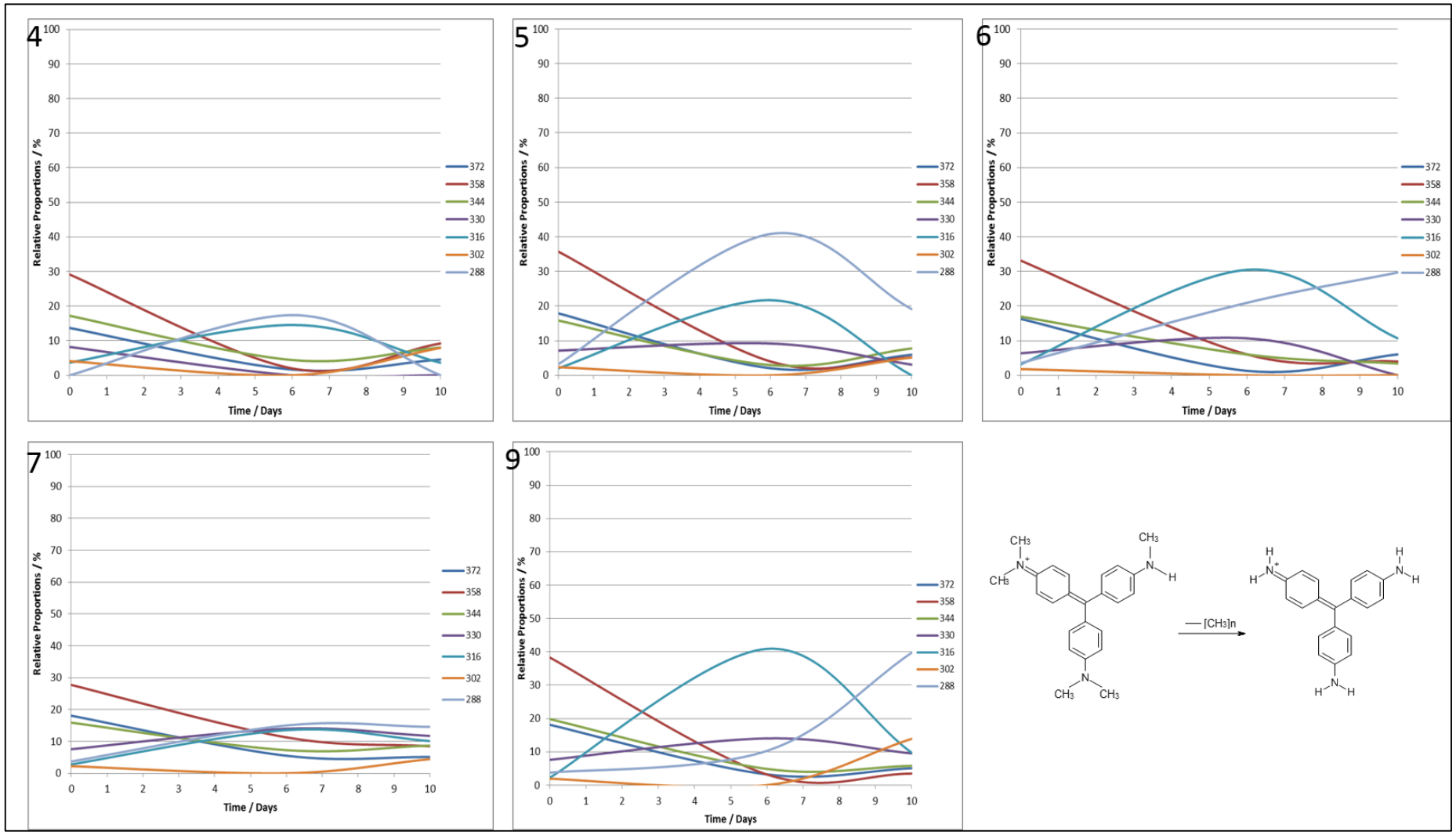


Figure 5.3 Aging Curves for Basic Violet dyes in Dye mixtures 4, 5, 6, 7 and 9 during Photofading

Relative ion count data for m/z 372 and 358 for all five black mixtures are displayed in Table 5.4. It is clear to see that both dyes reduced significantly in relative proportion over the ten days.

Table 5.4 Table to show Relative Proportions from Ion Count Data for m/z 372 and 358 for Mixtures 4, 5, 6, 7 and 9 after Photofading

Mixture	M/z 372		M/z 358	
	T = 0	T = 10	T = 0	T = 10
4	30	8	63	15
5	50	8	100	7
6	50	7	100	5
7	19	7	28	12
9	47	12	100	8

The aging curves for the benzophenone degradation products for dye mixtures 4, 5, 6, 7 and 9 after photofading are displayed in Figures 5.4. For all mixtures the products generally increased in relative proportion over the 10 days as degradation was taking place, but there do not appear to any significant results. The values were not particularly high which indicates degradation of the parent components was still taking place. This was consistent with the parent dye data.

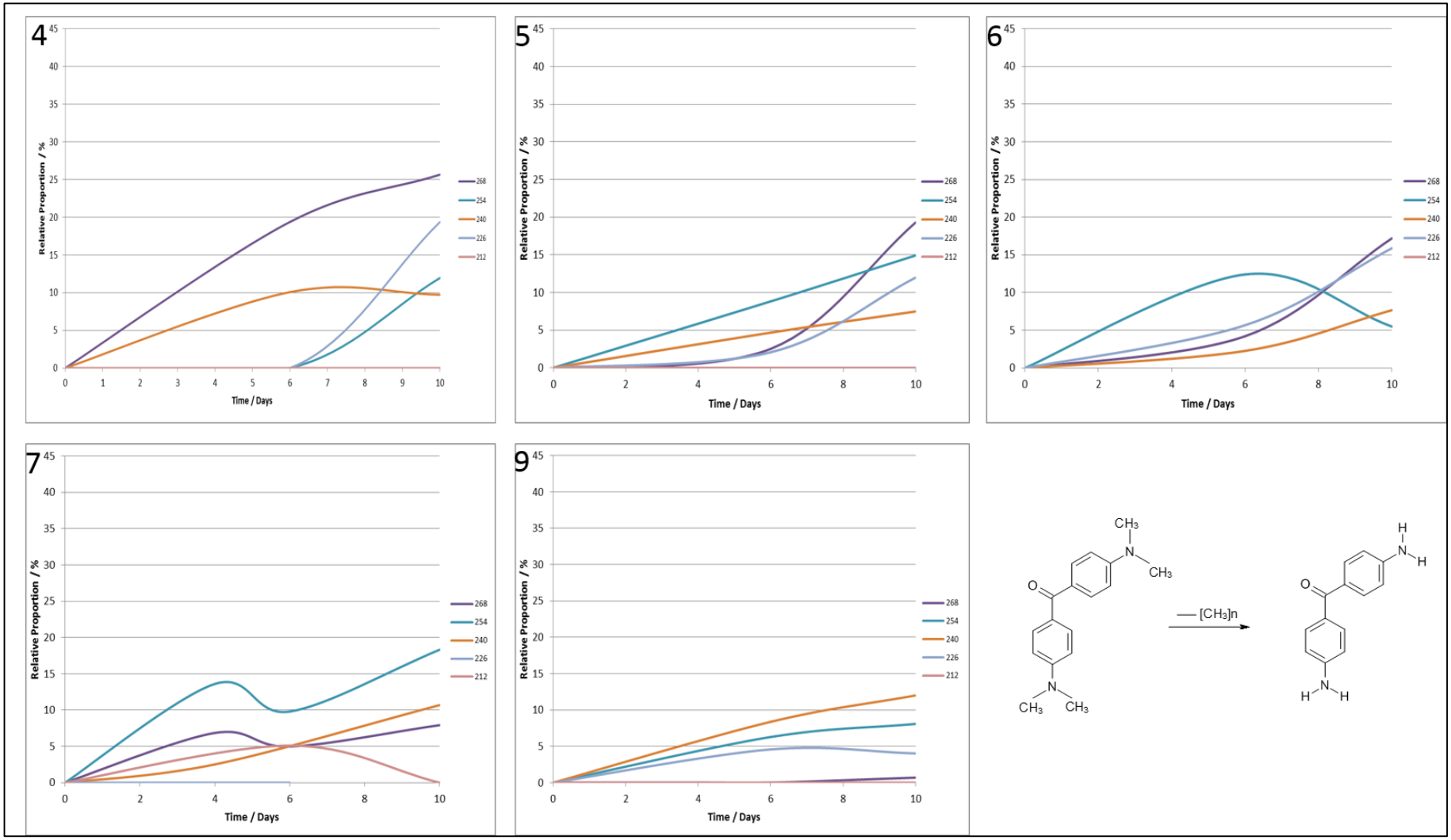


Figure 5.4 Aging Curves for Benzophenone Products from Basic Violet 3 in Dye Mixtures 4, 5, 6, 7 and 9 during Photofading

Table 5.5 shows the relative proportions of the component types in the dye mixtures when fresh and after 10 days based on the ion count data. The reduction in dye components over time can be seen, but the amount of change is different for each mixture with mixtures 4 and 5 having the greatest reduction in dyes and increase in benzophenones. For all mixtures the increase in benzophenone components was significant, in most cases doubling in proportion.

No phenolic compounds were detected in high enough quantities to confirm their existence for any of the mixtures.

Table 5.5 Table to show the Relative Proportions of Products based on Ion Count Data in Dye Mixtures after Photofading

	Dyes /%		Benzophenones /%		Phenols /%	
Mixture	**T = 0**	**T = 10**	**T = 0**	**T = 10**	**T = 0**	**T = 10**
4	81	33	19	67	0	0
5	85	46	15	54	0	0
6	83	54	17	46	0	0
7	90	75	10	25	0	0
9	92	87	8	13	0	0

5.2.3 Photofading of Inks on Paper

The ink samples were placed indoors against a window facing northeast for natural sunlight and they remained there until the ink had faded. The ink samples all degraded at different rates. Figure 5.5 shows what the black inks looked like to the unaided eye after 15 months using a Video Spectral Comparator 5000.

The black Micron sample appears to be the most stable of the black inks, which all appear brown in colour. The Papermate (Flexigrip and Comfort) and Bic brands were the least stable and by 20 months were not visible.

Figure 5.5 Image of Fresh Black Ink and ink exposed to daylight for 15 months

The VSC was used to look at the absorbance, reflectance and fluorescence of the samples before and after photodegradation. No changes were identified for any inks for IR absorbance or reflectance. Figure 5.6 shows the results from fluorescence analysis of black ink samples after 23 months.

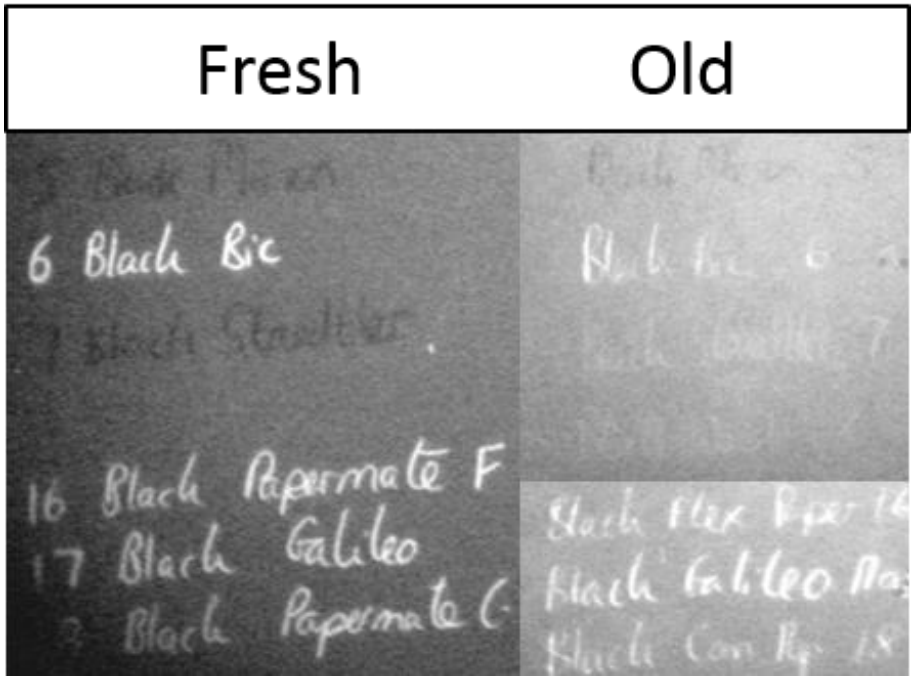


Figure 5.6 VSC Fluorescence images for Black Pen Samples (Fresh and Old)

The samples that fluoresced when fresh were 6, 16, 17 and 18. What can be seen in Figure 5.6 is that when the samples start to degrade and fade the fluorescence characteristics can change. The intensity of the fluorescence was less for the same samples over time, but fluorescence has been induced for samples 7 and 8 when old, but not when fresh. Sample 5 still did not fluoresce. Also under visible light, samples 6, 16 and 18 no longer had any colour and were virtually unreadable. Using the VSC, the fluorescence of these samples allowed the writing to be visualised which is a novel approach for ink analysis.

There was not one common dye that was present in all of the samples that fluoresced, but Basic Blue 9 was present in samples 7, 8, 16 and 18. It belongs to the group of thiazine dyes due to its central bond of four carbon, one sulphur and one nitrogen as shown in Table 1.2. Basic Blue 9 is known to fluoresce when oxidised, but it is only recently that it was found to luminesce in its reduced colourless form.[155] This may explain the fluorescence for these samples but further investigation is needed in this area to determine what is causing the fluorescence to develop in some samples after photofading. This could be extremely valuable information as it could be used to re-image any ink that contains that specific fluorophore without chemical intervention.

If faded writing has been written over with a different ink, this technique may be able to re-visualise the original ink. This method is used in document examination to detect alterations and additions, but it has not been reported for the use of faded inks.

Ink samples were analysed using APCI-MS over a 23 months and one example of the typical mass spectra produced of a black ink (pen 16-Galileo) is given in Figure 5.7

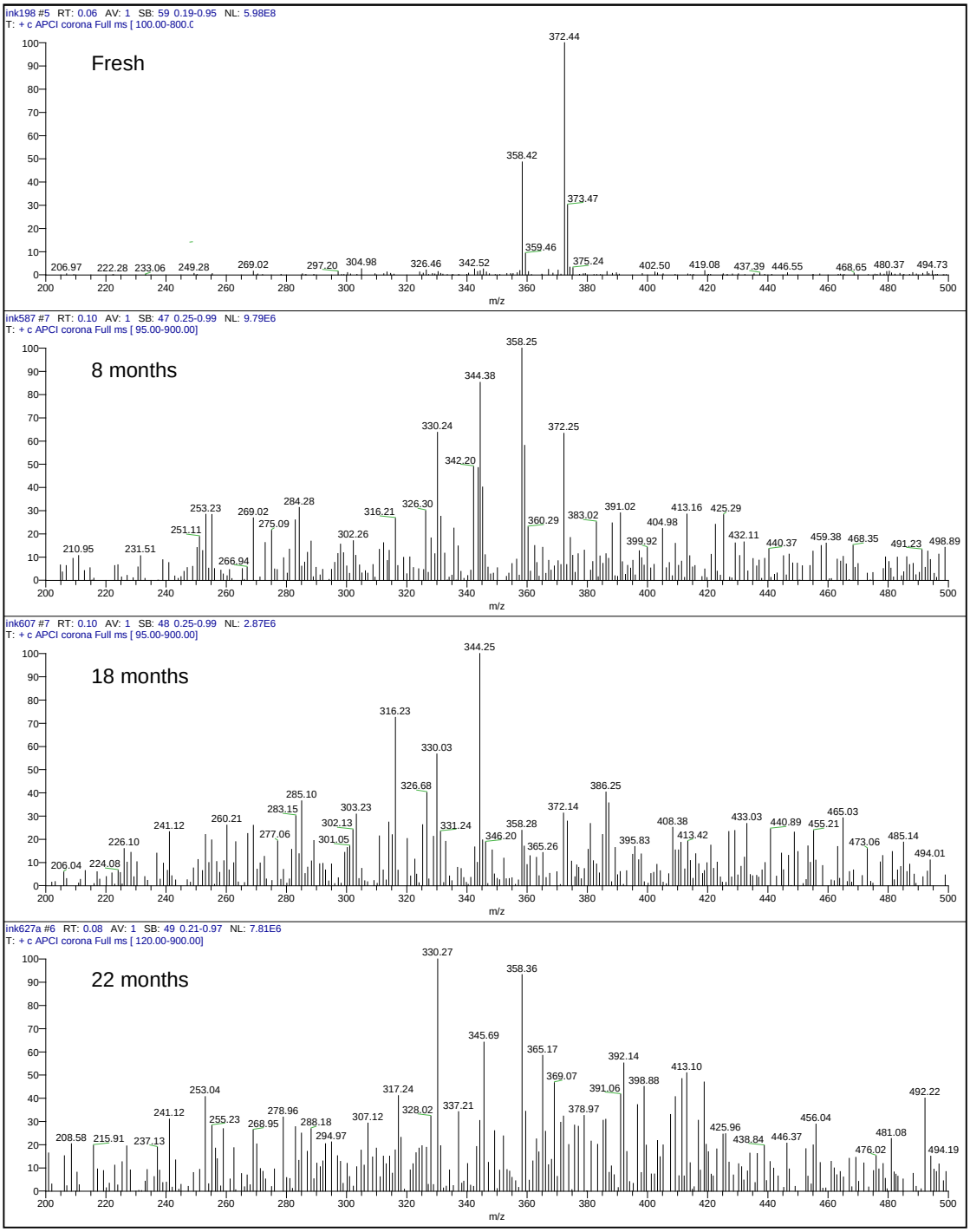


Figure 5.7 APCI-MS Spectra of pen 16 over 22 months exposure to sunlight

The aging curves displaying the relative proportions of the Basic Violet 3 and 1 dye components in all seven black inks are shown in Figures 5.8 and 5.9. Visually Bic and Papermate were the most faded of the inks, but it was difficult to tell this from the aging curves as there was a lot of activity for all of the inks and no real pattern emerged. Discrimination between the pens would be difficult based on this data.

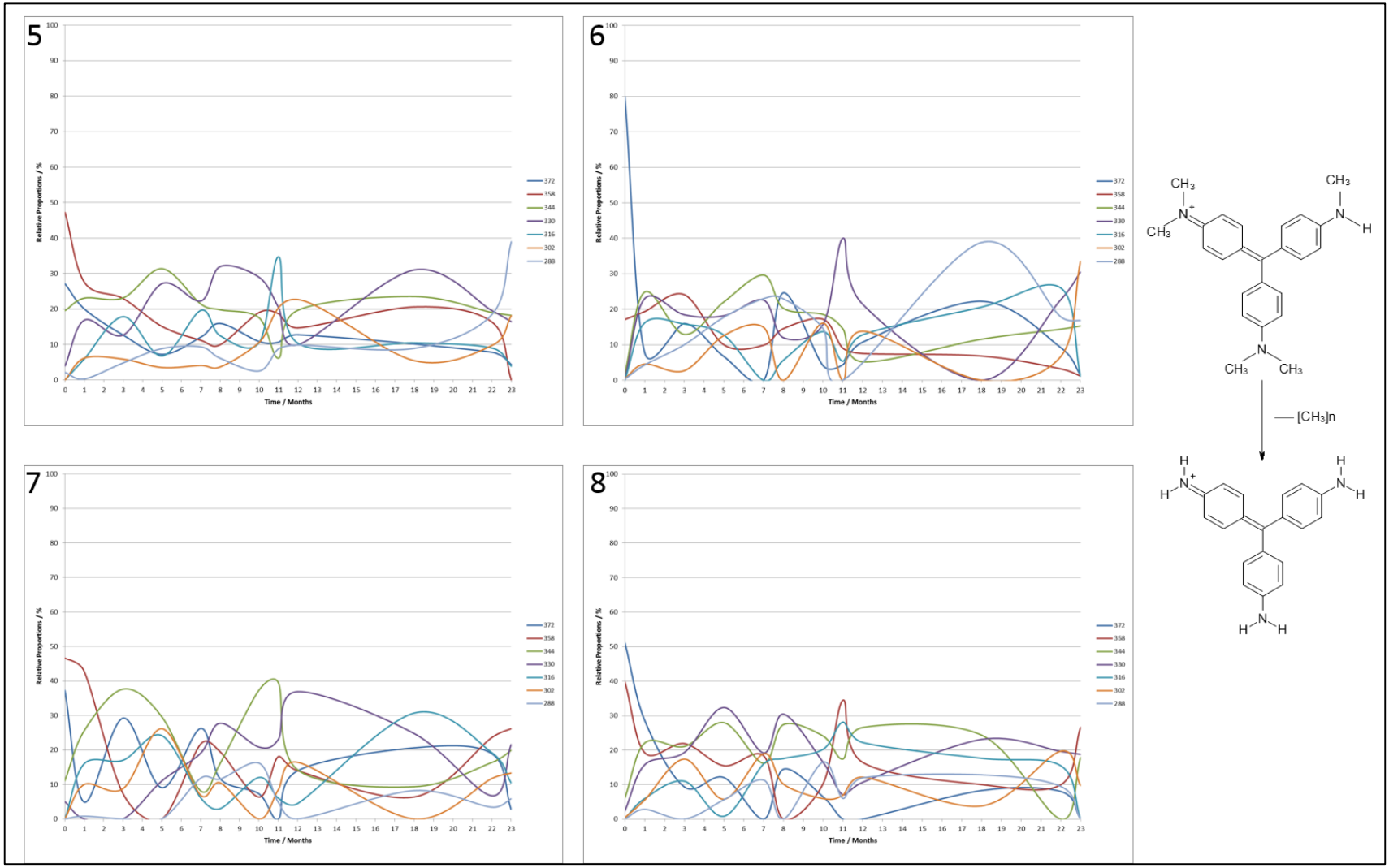


Figure 5.8 Aging Curves for Basic Violet 3 in Black Pens 5:Micron, 6:Bic, 7:Staedtler, 8:Woolworths after 23 months exposure to sunlight

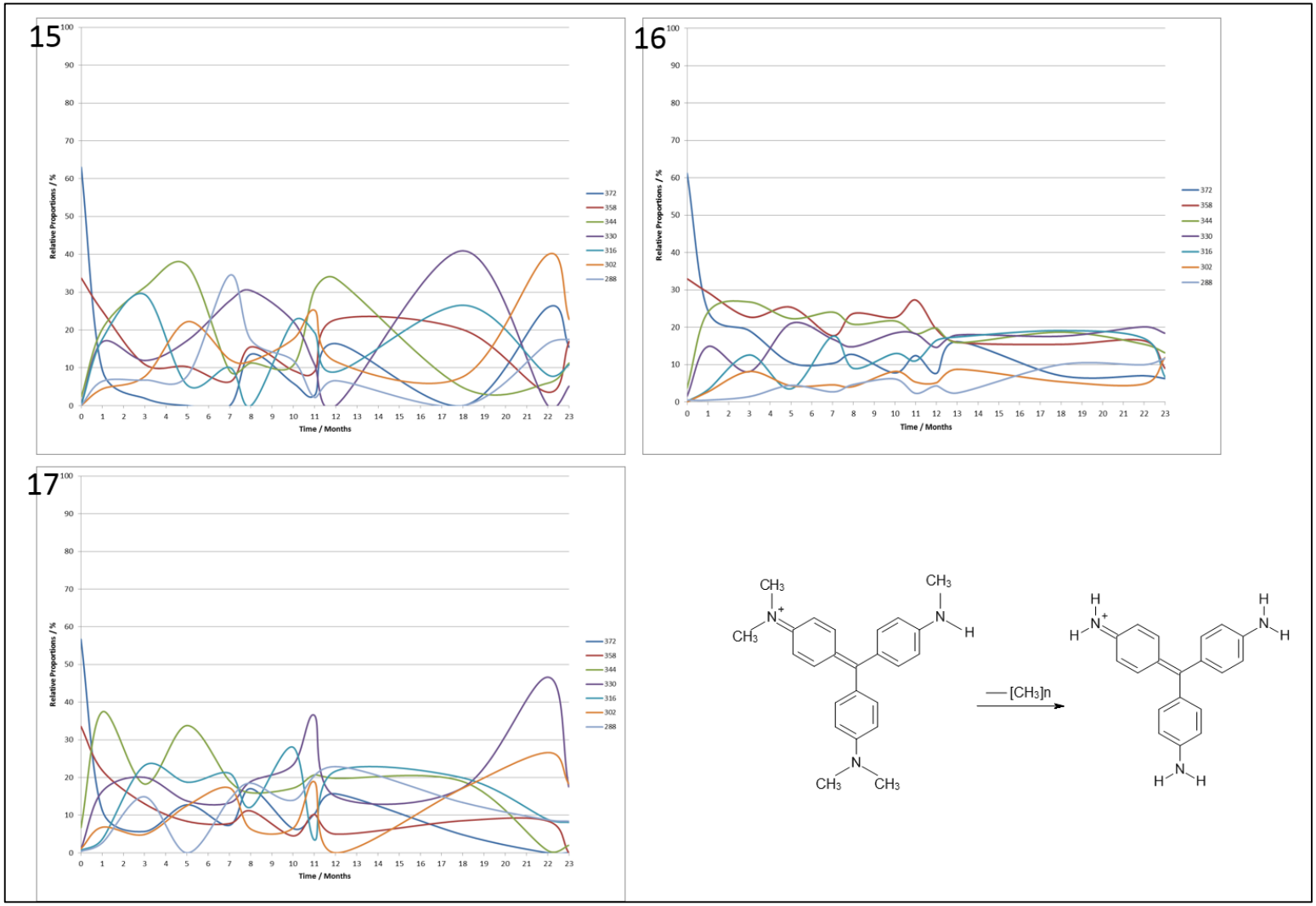


Figure 5.9 Aging Curves for Basic Violet 3 in Black Pens 15: Papermate Flexi, 16: Galileo, 17: Papermate Comfort after 23 months exposure to sunlight

There was a fluctuating pattern present in all of the pens which signifies the continual production and degradation of each of the homologue components, but the points at which different homologues peak was different for each pen. Also there was no clear single product that dominated over time. This shows that the degradation of Basic Violet dyes was not a simple successive process but what appears to be more random for each pen. Whilst there was no clear pattern to the degradation of all the pens, it was clear that discrimination between the pens was possible as the degradation for all pens was completely different. Table 5.6 shows the difference between relative ion count data for m/z 372 and m/z 358 for the seven black pens over the 23 months. These figures also allow some discrimination between the pens and show the degree of degradation. From this data it appeared that all the inks underwent significant degradation, but pen 6 (Bic) had the greatest degree of degradation for m/z 372 which supports the visual observation. Discrimination using ion count data was possible for some pens, but no discrimination could be done between pens 8, 15, 16 and 17.

Table 5.6 Table to show ion count data

Pen	M/z 372		M/z 358	
	T = 0	T = 23 months	T = 0	T = 23 months
5	57	7	100	6
6	100	0.25	21	0.2
7	79	0.6	100	6
8	100	6	77	4
15	100	5	53	6
16	100	4.5	53	6.5
17	100	3	59	3

5.2.4 Comparison of Fading with No Light

As shown in previous sections, degradation occurs in the presence of light, but it could also occur without light. All ink on paper samples were stored in a folder and analysed in the same way as the light samples to determine if the light had an effect. Figure 5.10 shows the APCI mass spectra of pen 16 (Black Galileo) stored in a folder at room temperature for 22 months. The spectra revealed no

change for the sample between fresh entries and after 4 months in the dark. In contrast, after 1 month exposure to daylight, strong signals of degradation products of Basic Violet 1 were observed. No measurable change was found for the degradation products of Basic Violet 1 even after 22 months stored in the dark. These results were consistent with all other samples. These results are in agreement with the facts mentioned previously that the natural aging of ink entries is accelerated when the samples are exposed to daylight through a window, relative to samples not exposed to light.

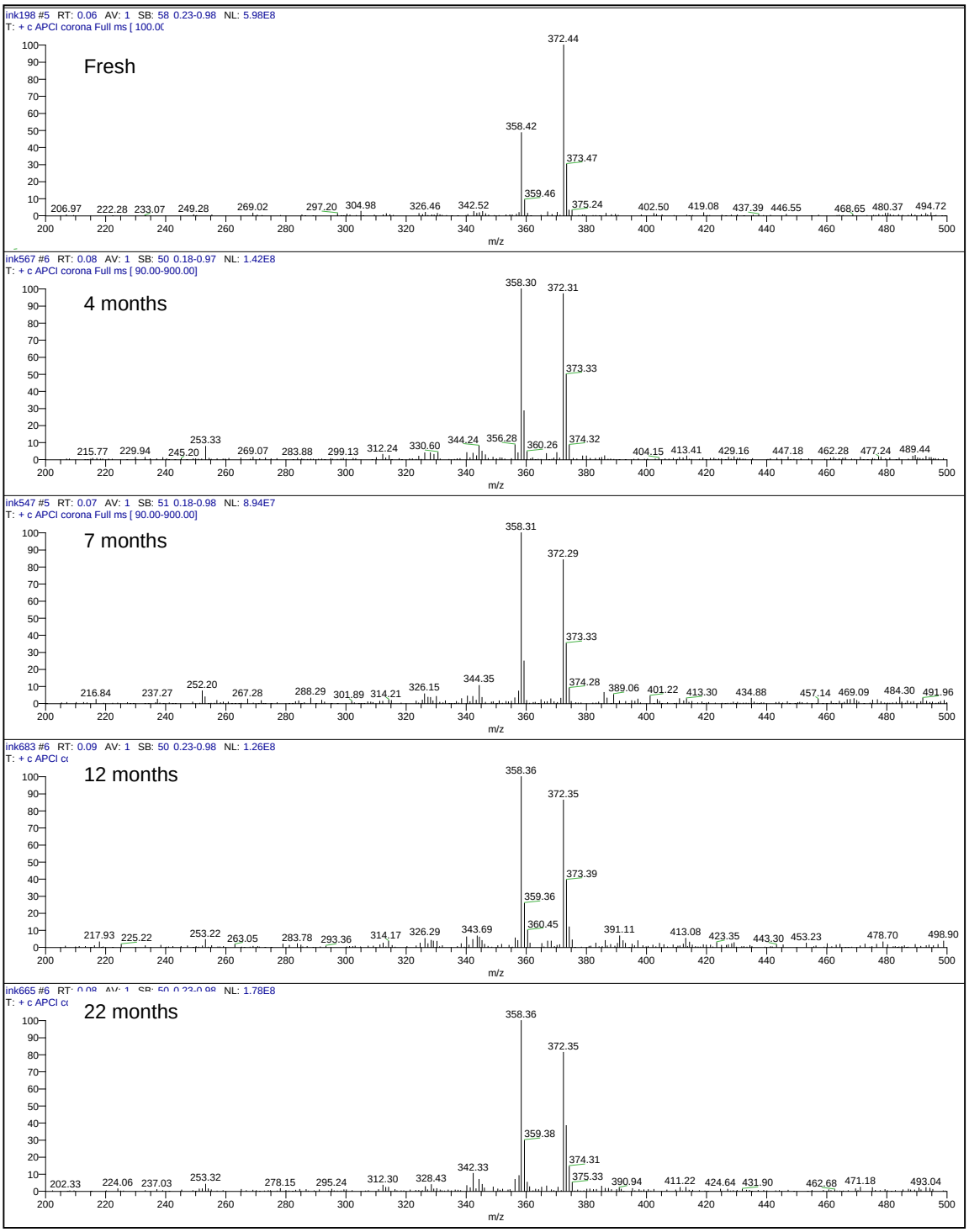


Figure 5.10. APCI-MS Spectra of Pen 16 stored in a folder for 22 months

Figure 5.11 shows the direct comparison for Basic Violet 3 in pen 16 with and without light exposure over 23 months. It proves that light does indeed accelerate the degradation process. M/z 372 and 358 had the highest relative proportions which remained so over the 23 months in the dark, which shows that these dyes were actually quite stable when there were no external influences such as light. A minimal amount of degradation in the dark has occurred over 23 months, as

the RPs did change slightly, but as not all degradation components were even detected, the degradation was far from complete.

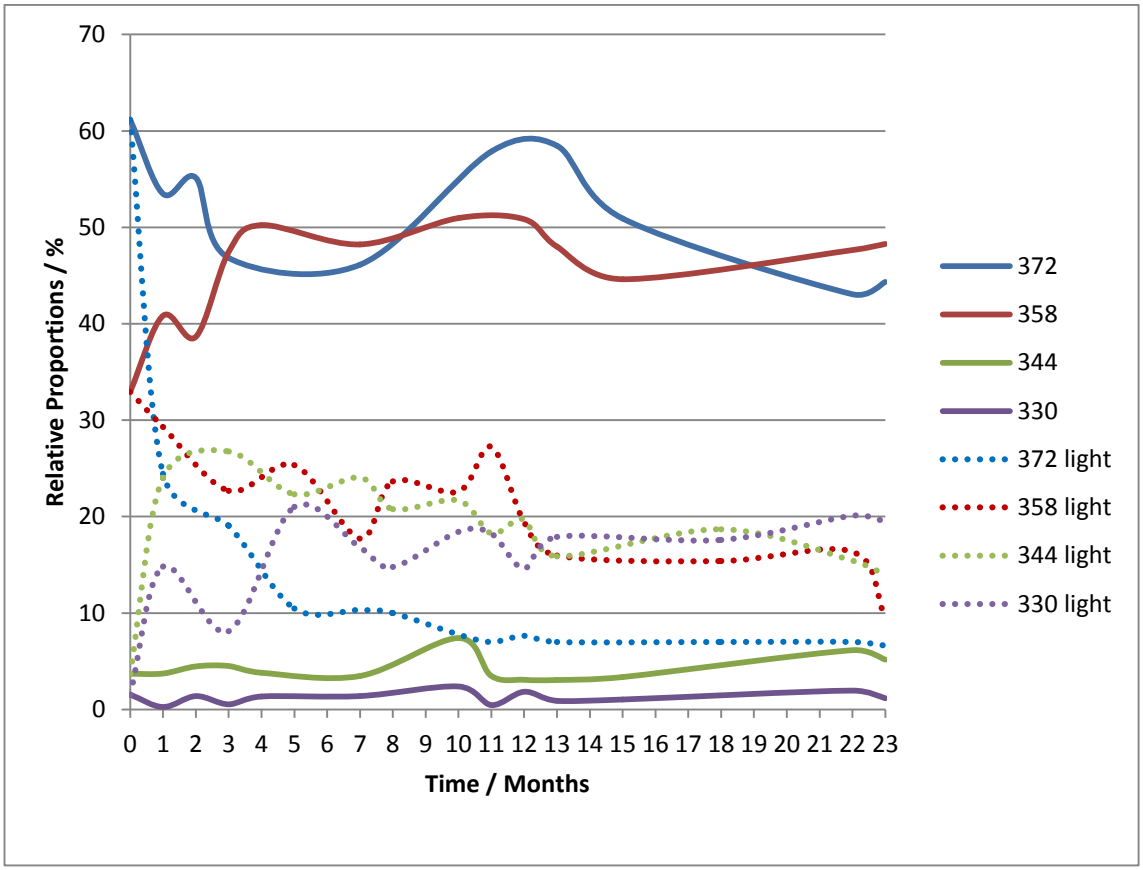


Figure 5.11 Aging Curves for Pen 16 – Basic Violet 3 with and without light exposure for 23 months

5.3 Bleaching with Hydrogen Peroxide

5.3.1 Bleaching of Dye Standards

Each dye standard had 2 µl of 5% H_2O_2 added to it before being monitored by APCI-MS. Samples were kept in the dark or subjected to artificial daylight over six days to initiate the degradation process of bleaching.

5.3.1.1 In the Dark

The standard dye solutions were stored in a cupboard during this stage of analysis in order to be able to compare with samples subjected to artificial daylight. The aging curves for Basic Violet 1 and 3 are shown in Figure 5.12. For Basic Violet 3 (graph a) the molecular ion (m/z 372) decreased quickly to a relative proportion of 10% and the subsequent degradation homologue (mono-N-

demethylated) m/z 358 increased. Similarly for Basic Violet 1, (graph b) m/z 358 decreased and the subsequent homologue m/z 344 increased. At T = 0 the relative ion count for the molecular ion m/z 372 was 100%, but at T = 6 days it had dropped to just 2.4% which was a significant decrease which could indicate a rapid degradation of the dye in just six days.

The degradation of Basic Violet 1 appears to be quicker than Basic Violet 3 as the dye homologues were being produced earlier and in greater quantity. At T = 0 the relative ion count for the molecular ion m/z 358 was 100% and at T = 6 days it had dropped to 16%. This was slightly higher than for Basic Violet 3 at 2.4%, but as Basic Violet 1 also contains some Basic Violet 3, during the demethylation process m/z 358 will be produced simultaneously by m/z 372 whilst it is also degrading.

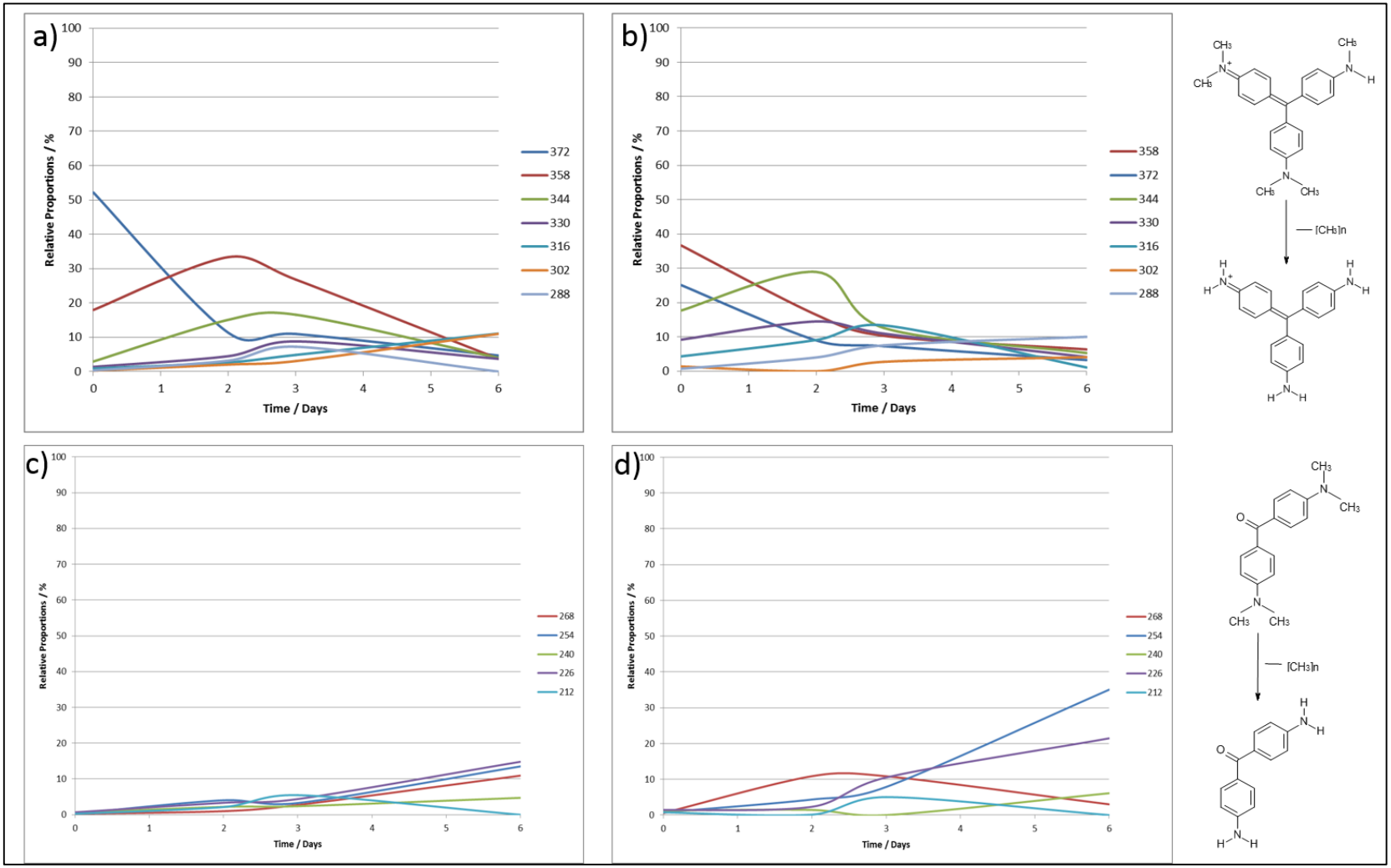


Figure 5.12 Aging Curves for dye standards a: Basic Violet 3 b: Basic Violet 1, c: Benzophenones for BV3 d: Benzophenones for BV1 during treatment with hydrogen peroxide

Benzophenone products were also detected after treatment with peroxide as shown in Figure 5.12 graphs c and d. In Basic Violet 3 all products except the fully demethylated product m/z 212 gradually increased over time. At T = 0 the relative ion count for the molecular ion m/z 268 was 0%, but at T = 6 days it had risen to 5%, however the predominant benzophenone at T= 6 days was m/z 226 with a value of 7.6%.

For Basic Violet 1 shown in graph d, m/z 254 was produced in a significant amount compared to the other products as the RP increased from 0 to 35%. This may be explained as it is formed from the loss of 1 -CH_3 group, which can be from either side of the molecule's structure which would double the possibility of it being produced. At T = 0 the relative ion count for the molecular ion m/z 268 was 0 and at T = 6 days was 7.6%. At T = 6 days the most abundant benzophenone was the tri-methylated ion m/z 254 with a value of 89%. These results are consistent with the rate of degradation being quicker for Basic Violet 1 than Basic Violet 3 as the values for the benzophenone products were higher.

Phenol degradation products were detected for Basic Violet 3 from 2 days where the di-methylated product m/z 137 had a relative count of 7.5%. At six days m/z 137, 123 (mono-methylated) and 109 (fully demethylated) had relative counts around 3%. No phenol products were detected at six days for Basic Violet 1, but they were detected at two and three days with relative counts of 5% for m/z 137 and 2% for m/z 123. This would indicate that they were produced quickly, but were unstable and broke down. This would corroborate the theory of faster degradation of Basic Violet 1.

Table 5.7 shows the relative proportions based on ion count data of the component types when fresh and after six days. It clearly shows the reduction in dye components to at least half their original proportion and shows the increase in contribution of the degradation products; benzophenones and phenols, with the benzophenones most abundant after six days.

Table 5.7 Table to show the Relative Proportions of Products based on Ion Count Data after Treatment with Hydrogen Peroxide

	Basic Violet 3		Basic Violet 1	
	T=0	**T=6**	**T=0**	**T=6**
Dye /%	74	38	77	35
Benzophenones /%	24	44	20	65
Phenols /%	2	17	2	0

Figure 5.12 showed that even a small amount of hydrogen peroxide can induce aging in the dye standards and it was almost immediate as the effects were rapidly detectable with big changes occurring within two days. The values for m/z 372 and 358 in Basic Violet 3 and 1 had dropped significantly over the 6 days to only 5% and the lower homologues such as m/z 316 and 302 were already increasing at 2 days. There is published research on dye degradation through photochemical processes with the use of hydrogen peroxide,[65,152,153] but there is limited information on the effects without light. These results provide some evidence of de-methylation taking place without light. Benzophenone products were also produced for these dye standards providing further evidence of degradation and more specifically of cleavage of the central carbon bond as described earlier. It is clear to see that hydrogen peroxide does have a role in the de-methylation process with or without light.

5.3.1.2 In the Light

In addition to the use of hydrogen peroxide to treat the dye standards, these samples were also subjected to artificial daylight (as detailed in Chapter 2) to see what affect it would have on the dyes as. The results are shown in Figure 5.13. For the dye Basic Violet 3, m/z 372 decreased quickly for two days from a relative proportion of 40% to around 5%. M/z 372 breaks down into m/z 358 which would explain why after three days the aging curve showed an increase for m/z 358. M/z 302 and 288 increased over the time period, with m/z 302 having the highest RP of 11% after six days. With just peroxide, m/z 302 also had the largest RP after 6 days and it was also 11%. M/z 330 and 344 did not show significant changes in RP remaining below 3%. Here m/z 316 increased to 11% at 3 days,

but when just peroxide was used it did not reach this value until 6 days. At T = 0 the relative ion count for m/z 372 was 100%, but at T = 6 days it had dropped to 2%. All dye homologues were also low in value at six days, m/z 302 (penta-demethylated) was the most abundant increasing in ion count from 0 to 6%.

For Basic Violet 1, as shown in graph b, m/z 358 and 372 decreased sharply in RP for two days to around 5%. The later homologues m/z 330 (tri-methylated) and 316 (di-methylated) increased in RP for two days to peak at 11% and 16% respectively, but with light m/z 302 and 288 both showed significant increases in RP values (16% and 24% respectively) not seen without light. The most abundant component varied over time, but there was evidence of successive demethylation as m/z 316, then m/z 302 and finally m/z 288 were the most abundant one after the other. At T = 0 the relative ion count for the molecular ion m/z 358 was 100% and at T = 6 days it had decreased to 1.5%. The most abundant homologue at six days was the tri-demethylated product m/z 316 with an ion count of 7%.

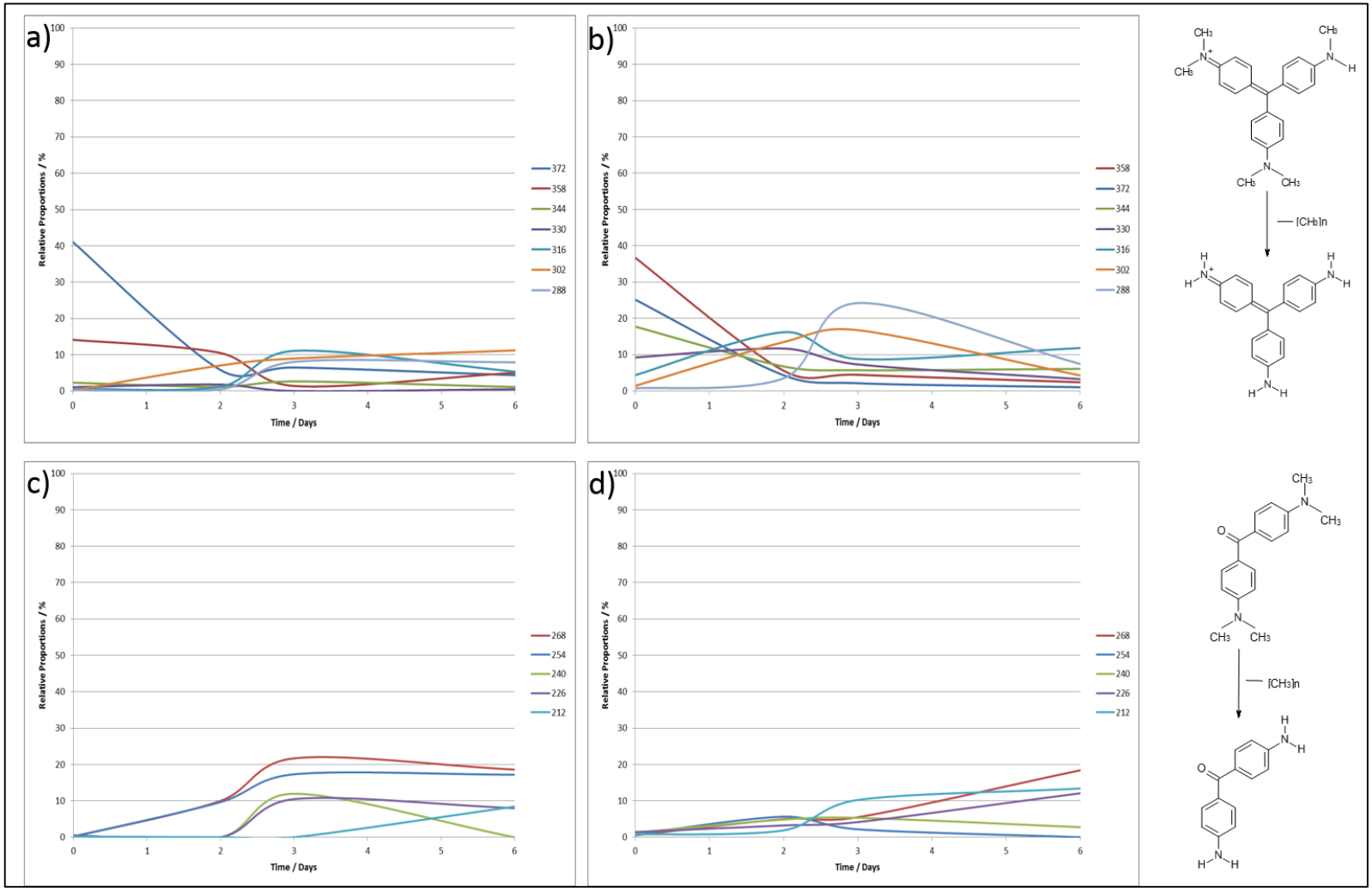


Figure 5.13 Aging Curves for dye standards a: Basic Violet 3 b: Basic Violet 1, c: Benzophenones for BV3 d: Benzophenones for BV1 during treatment with hydrogen peroxide and light

Benzophenone products were also produced under these conditions as shown in Figure 5.14 graphs c and d. With Basic Violet 3, the RP values for all products were greater than without light indicating that degradation is influenced by light and the breakage of the central carbon bond in the main dye structure does occur. At T = 0 the relative ion counts for the molecular ions m/z 268 and 254 were 0% and at T = 6 days they had risen to 10% and 9.5% respectively.

For Basic Violet 1, the RP values were lower with light than without light but at T = 0 the relative ion count for the molecular ion m/z 268 was 0% and at T = 6 days it had risen to 11%. However it was at T = 2 days that all benzophenone products had relative counts of between 8% and 78%, indicating they were produced early and after two days they were probably degrading themselves.

Phenol products were not detected until six days for Basic Violet 3; m/z 137 (di-methylated) and 109 both had relative counts of 3%. For Basic Violet 1, products were detected at 2 days. M/z 137 had a relative count of 9% which decreased to 2% at six days. These results indicate that phenol products were produced with peroxide and light, but in extremely low quantities.

Table 5.8 shows the relative proportions of the component types when fresh and after six days based on ion count data. It clearly shows the reduction in dye components which was greater than without light showing that light has enhanced the rate of degradation. The proportions of benzophenones and phenols have increased particularly for Basic Violet 1. Without light, no phenols were detected at six days, but with light the phenols contributed to 11% of the composition. The benzophenone values recorded were slightly less with light for Basic Violet 1, but this can be explained as they have degraded into the phenol products.

Table 5.8 Table to show the Relative Proportions of Products based on Ion Count Data after Treatment with Hydrogen Peroxide and Light

	Basic Violet 3		Basic Violet 1	
	T=0	**T=6**	**T=0**	**T=6**
Dye /%	74	35	77	42
Benzophenones /%	24	52	20	46
Phenols /%	0	12	0	11

In order to determine if light in conjunction with peroxide has an impact on the degradation of the dye standards compared to no light exposure, comparative aging curves were produced and they are displayed in Figures 5.14. For all the black dye standards, the relative proportion values were much lower for all components when light was used in combination with hydrogen peroxide which was not expected. It was anticipated that light would enhance the degradation and values would be higher. Without the light, increases in components were seen which would correspond to their successive production following the degradation of homologues.

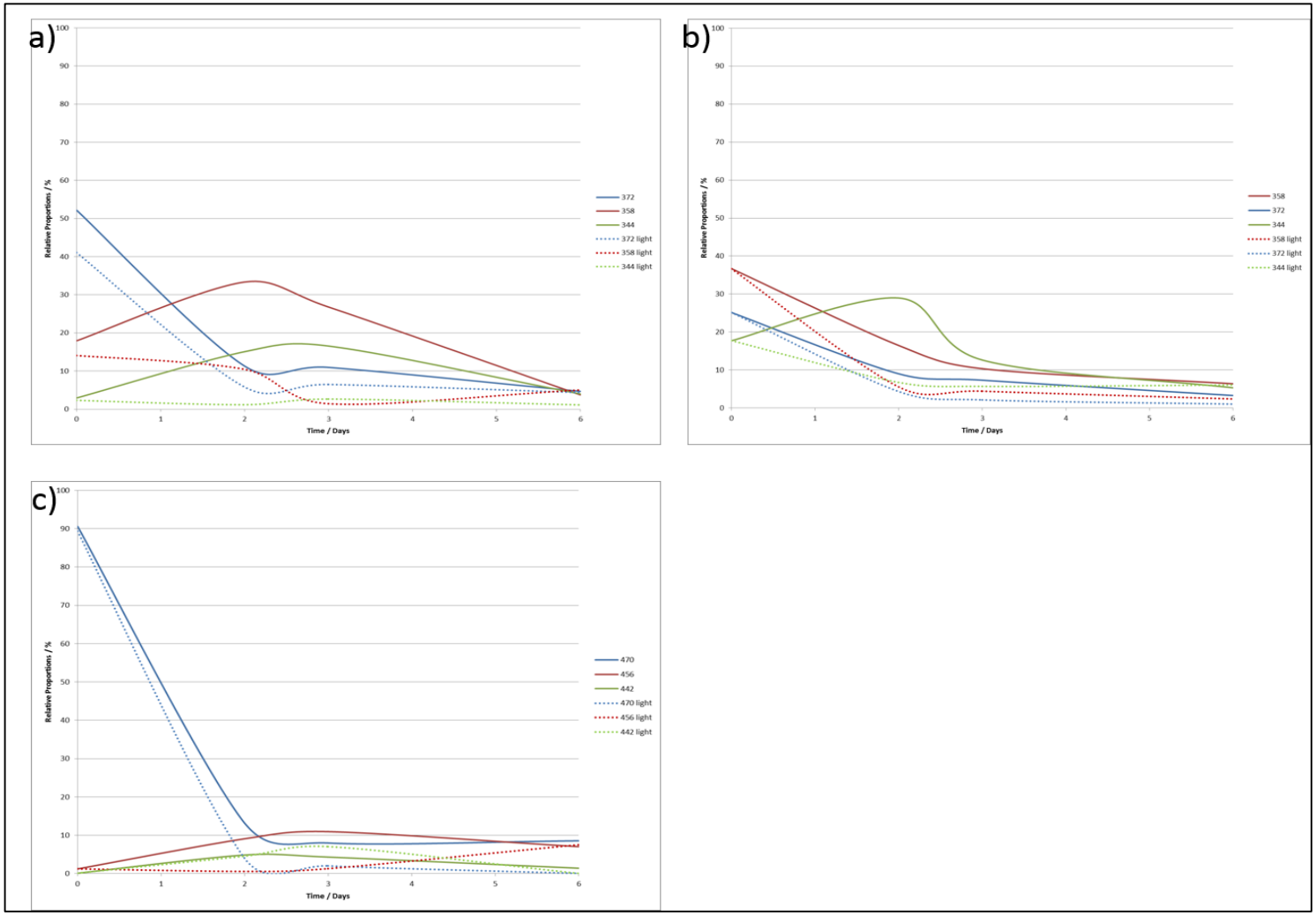


Figure 5.14 Aging curves for dye standards a: Basic Violet 3, b: Basic Violet 1 and c: Basic Blue 26 during Treatment with Hydrogen Peroxide with and without light

These results have shown that hydrogen peroxide has an impact on the degradation of dye compounds and in conjunction with light, the degree of degradation was quicker but the products were not formed in the same way or in the same proportions. It is important to find out if this will be the same when dyes are used in combination and when inks are on paper.

5.3.2 Bleaching of Dye Mixtures in Solution

By using dye combinations identified from the ink compositions of the ballpoint pen samples shown in Table 3.1, five dye standard combinations were produced as displayed in Table 5.9 for analysis to replicate ink degradation by hydrogen peroxide. APCI-MS analysis was carried out over ten days and aging curves produced of the results as show in Figure 5.15. Total ion counts were also used to determine the degree of fading.

Table 5.9 Dye Standard Dyes

Dye Standard Combination	Dye standards proportions	Pen Colour	Pen number
4	BV1 100 : BV3 40	Black	5
5	BV3 100 : BV1 40	Black	6
6	BV1 100 : BV3 65	Black	7
7	BV1 100 : BV3 85 : BR1 50	Black	8
9	BV3 100 : BV1 100	Black	15

The aging curves did not indicate a large degree of fading as the relative proportions for the degradation homologues did not really increase, and m/z 372 and m/z 358 only decreased slightly, however when the ratio of Basic Violet 1 was greater than Basic Violet 3, as in mixtures 4, 6 and 7, m/z 344 (4 Me) was produced in greater proportions suggesting the degree of degradation was greater. There was also no clear successive degradation through the homologues as would be expected. This may provide evidence of the competitive nature of the dyes and how they may compete for reactants and are able to quench degradation in others.

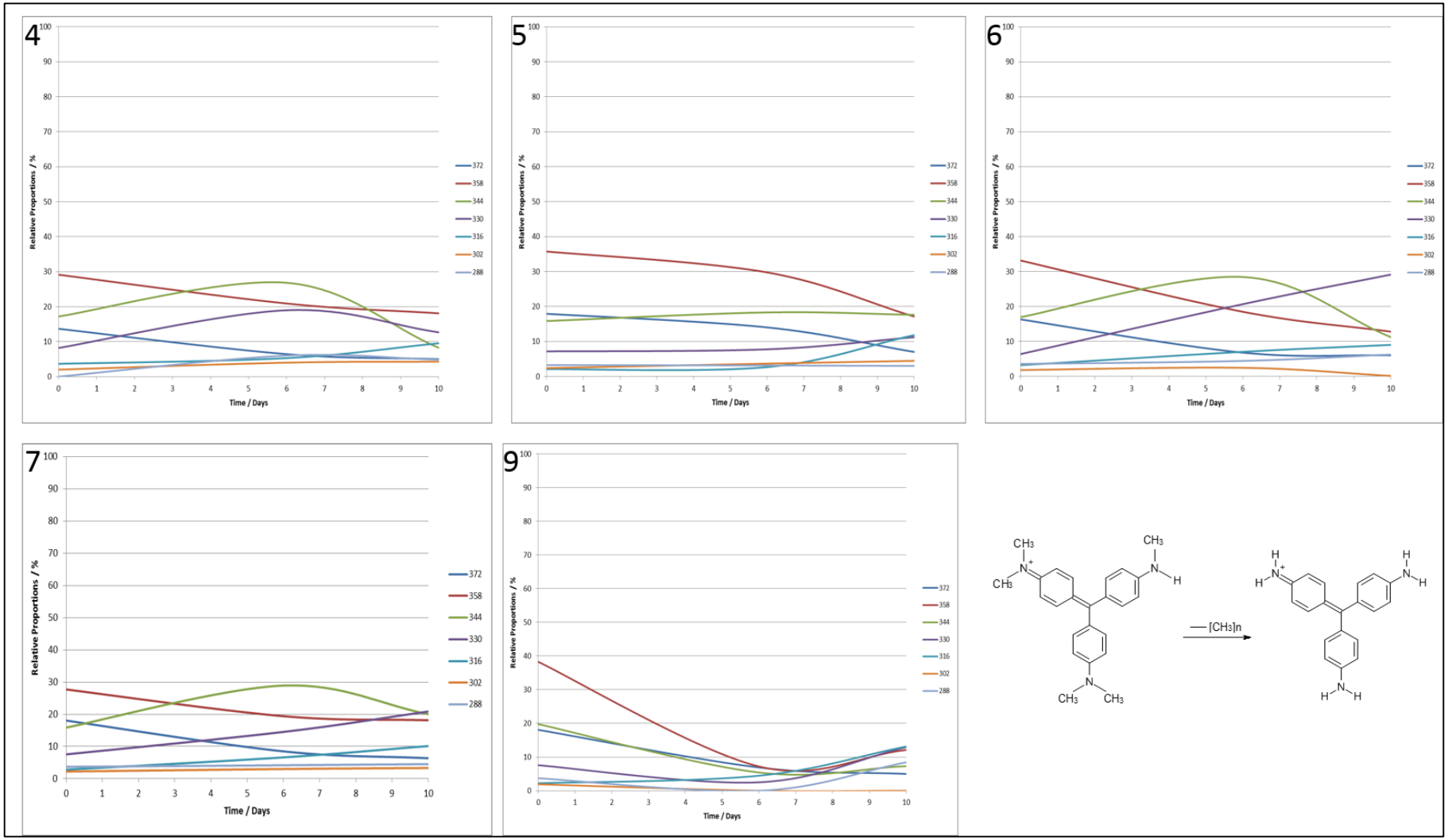


Figure 5.15 Aging Curves for Basic Violet dyes in Dye Mixtures 4, 5, 6, 7 and 9 during Treatment with Hydrogen Peroxide

The ion count data supports the theory of greater degradation by m/z 358 in each mixture compared to m/z 372 as shown in Table 5.10.

Table 5.10 Table to show the relative proportions of the dyes in the mixtures based on Ion Count Data after treatment with hydrogen peroxide

Mixture	M/z 372		M/z 358	
	T = 0	T = 10	T = 0	T = 10
4	30	22	63	34
5	50	10	100	25
6	50	18	100	18
7	18	3	28	8
9	50	10	100	10

Benzophenone products were also detected following hydrogen peroxide treatment (as shown in Figure 5.16). The aging curves were very different for each mixture. The greatest proportion of degradation products were present in mixtures 5 and 9 and they were produced earlier than in the other mixtures. Ion count data supported this with m/z 268 in mixture 5 reaching 34% proportion and m/z 240 reaching 13% in mixture 9. For the other mixtures, the products slowly increased over the 10 days, but quantities were low.

It appears that for the mixtures and hydrogen peroxide, the effects were slightly different to photofading. Ion count data showed greater reductions in dye components for photofading than bleaching.

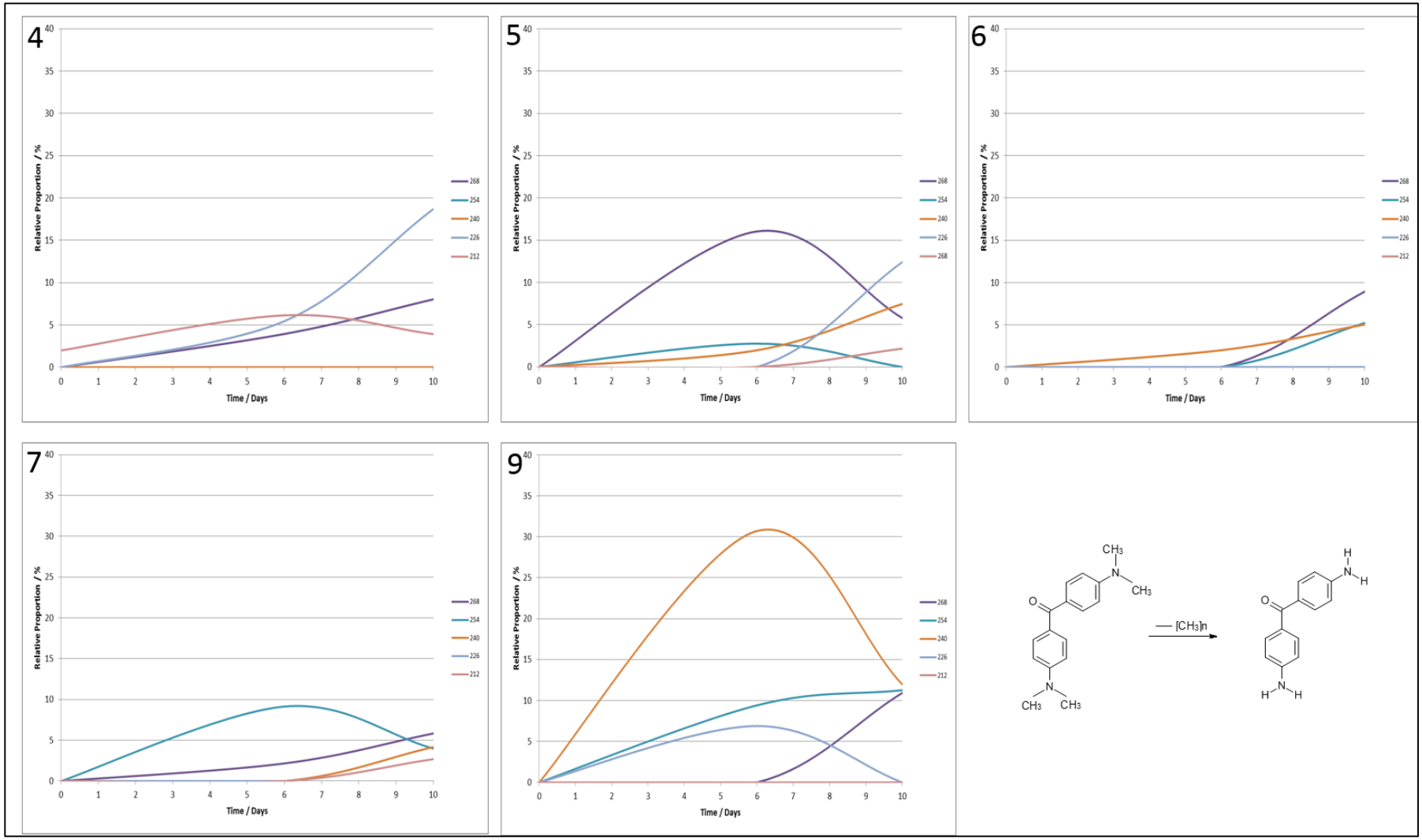


Figure 5.16 Aging Curves for Benzophenone Products from Basic Violet 3 in Dye Mixtures 4, 5, 6, 7, 9 during Treatment with Hydrogen Peroxide

No phenolic compounds were detected in high enough quantities to confirm their existence for any of the mixtures.

Table 5.11 shows the relative proportions of the component types in the dye mixtures when fresh and after ten days based on ion count data. The reduction in dye components over time can be seen, but these values were higher compared to the dye standards alone indicating less degradation as a mixture. Increases in the contribution of the degradation products benzophenones and phenols were observed, but once again the difference was smaller for the mixtures than the pure dye standards. The values for the phenol products were also significantly lower and not detected at all for some mixtures. The greatest degradation appears to be for mixtures 5 and 9.

Table 5.11 Table to show the Relative Proportions of Products in Dye Mixtures based on Ion Count Data after Treatment with Hydrogen Peroxide

	Dyes /%		**Benzophenones /%**		**Phenols /%**	
Mixture	**T = 0**	**T = 10**	**T = 0**	**T = 10**	**T = 0**	**T = 10**
4	81	69	19	30	0	0
5	85	69	15	31	0	0
6	83	79	17	20	0	0
7	90	95	10	5	0	0
9	92	61	8	33	0	0

5.3.3 Bleaching of Inks on Paper

Hydrogen peroxide was applied to the ink samples when written on the paper surface. Figure 5.17 shows the visible light results after three applications of hydrogen peroxide to all samples.

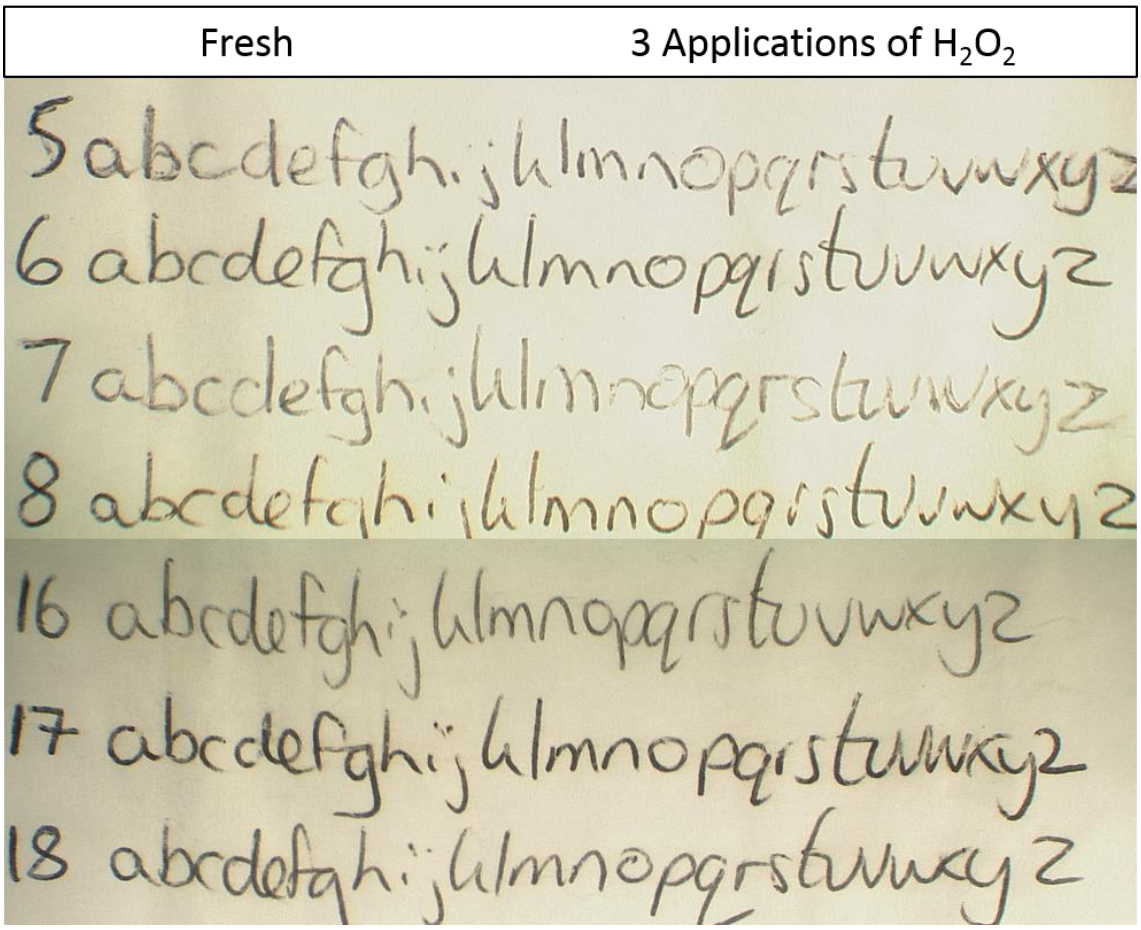


Figure 5.17 Image of Fresh Black Ink and Ink treated wiht 3 applications of Hydrogen Peroxide

After one application of peroxide, the inks were slightly lighter than without, but no significant fading had occurred. Peroxide was applied to the ink samples a total of ten times allowing them to dry between each application. Fading had occurred for all samples, but not to the same extent for each other. Sample 7 (Staedtler) showed the most significant fading with little colour remaining. For inks 8, 17 and 18, the colour remained in the ink, but the area surrounding the ink line changed colour to orange for sample 8 and yellow for 17 and 18. This was not evident for any other samples. It is widely acknowledged that when paper ages or is subjected to sunlight a “yellowing” effect will occur,[144,156] but it is unclear if the yellow surrounding the ink line for these samples is due to the peroxide reacting with the paper, or the peroxide reacting with solvent from the ink that is bordering the ink line commonly due to lateral diffusion.[45]

Fluorescence was also examined for the peroxide samples and the images are shown in Figure 5.18. Samples 6, 16, 17 and 18 exhibited fluorescence with and without peroxide treatment, but for pen 17 the level of fluorescence was reduced

post treatment, when there was fluorescence before. The remaining samples did not fluoresce before or after ten applications of peroxide treatment. Pen 17 was still black in colour after the peroxide treatment, so the lack of fluorescence was not due a lack of dye molecules. It could be due to a change in the dye structures and therefore a change in excitation wavelengths so that fluorescence does not occur.

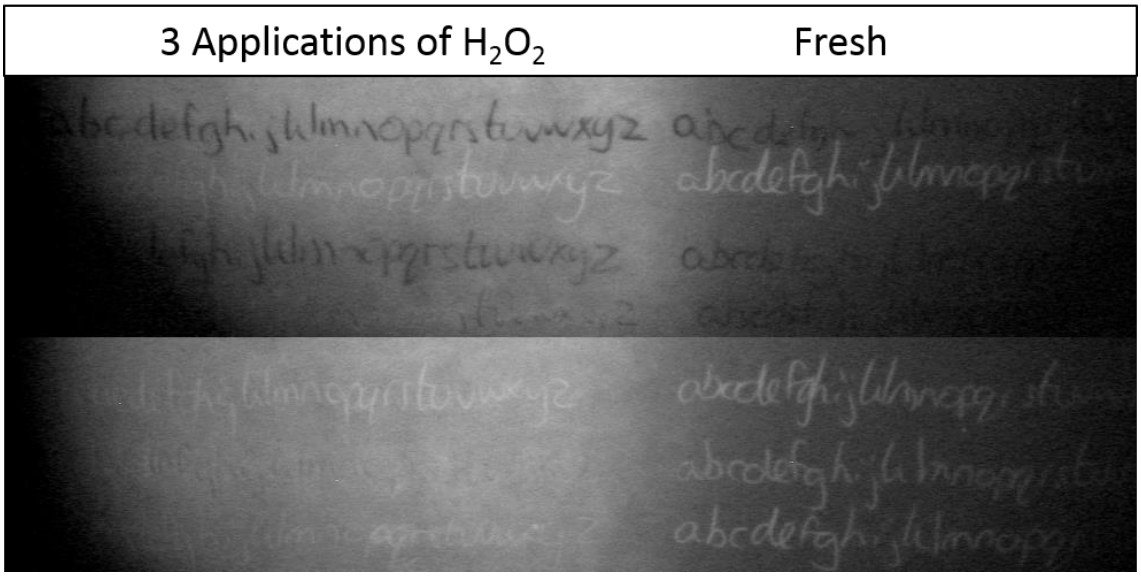


Figure 5.18 VSC Fluorescence of Black Pen Samples Fresh and after 3 applications of Hydrogen Peroxide

Figures 5.18 also shows that the area in which the peroxide has been applied to the paper appears brighter than the non-treated area.

These results were similar to photofading with regards to samples 6, 16, 17 and 18 exhibiting fluorescence before and after treatment, but with photofading samples 7 and 8 had fluorescence induced by photofading, and this was not seen from bleaching.

APCI-MS data was analysed over 10 applications to determine the aging curves of the black pen inks on paper. The aging curves for the main dye components can be seen in Figures 5.19 and 5.20. Staedtler visually was the most faded of the inks and this was corroborated with the aging curves. There was a lot of activity by the homologues and ion count data also supported this. At T = 0 m/z 372 had a relative count of 100% but at T = 10 applications it had dropped to 8% for ink 7.

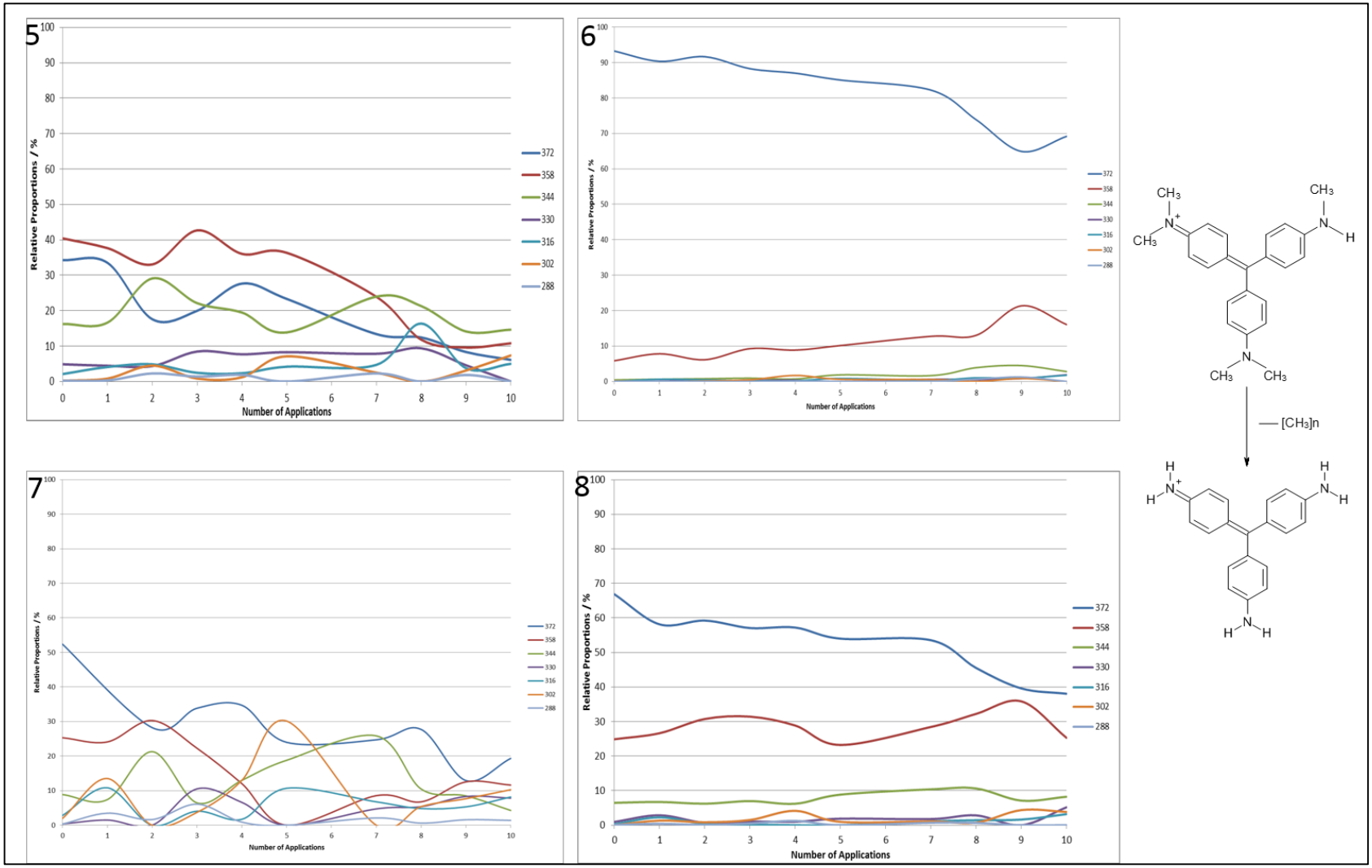


Figure 5.19 Aging Curves for Basic Violet 3 in Black Pens 5: Micron, 6: Bic, 7: Staedtler, 8: Woolworths after treatment with hydrogen peroxide

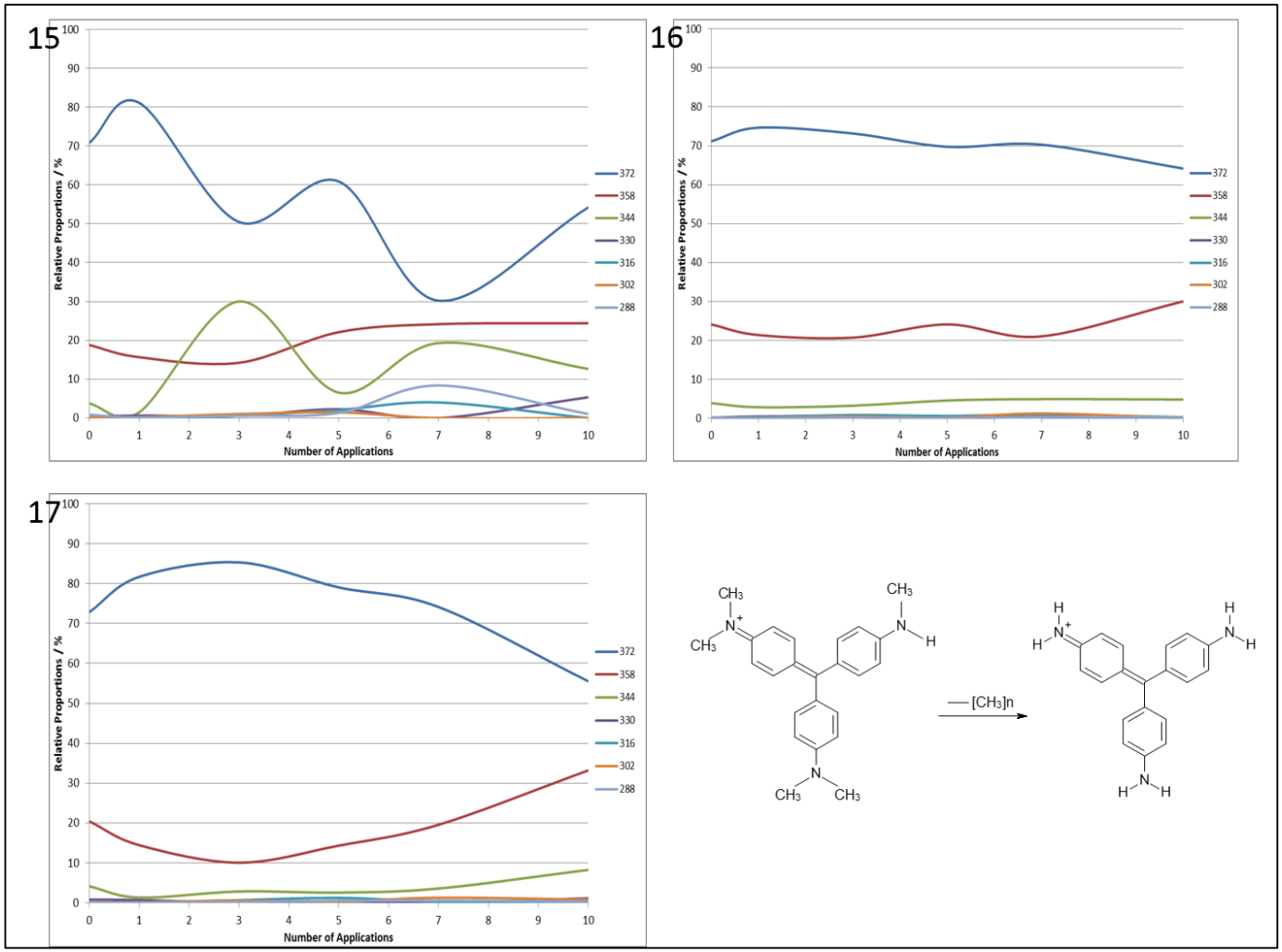


Figure 5.20 Aging Curves for Basic Violet 3 in Black Pens 15: Papermate, 16: Galileo, 17: Papermate after treatment with hydrogen peroxide

Ink 5 - Micron also displayed some activity with m/z 344 (4 Me), but m/z 302 (1 Me) was produced for Staedtler which is a later homologue. This would support greater degradation for Staedtler than Micron. Bic (pen 6) did not show much visual fading and this was supported by the aging curves. For pens, 6, 8, 16 and 17 there was very little degradation. For all of them ion count data for m/z 372 did not decrease from 100% for all 10 applications of peroxide, but there were subtle differences in the data for m/z 358 which allows the discrimination between the pens.

The black inks also produced benzophenone products as shown in Figures 5.21 and 5.22. Higher RP values were consistent with the data for the inks that had shown degradation for the parent dyes; 5, 7, and 15. The remaining samples did produce benzophenones, but in extremely low quantities and for pens 6 and 8 they were not significant values until after eight applications of peroxide.

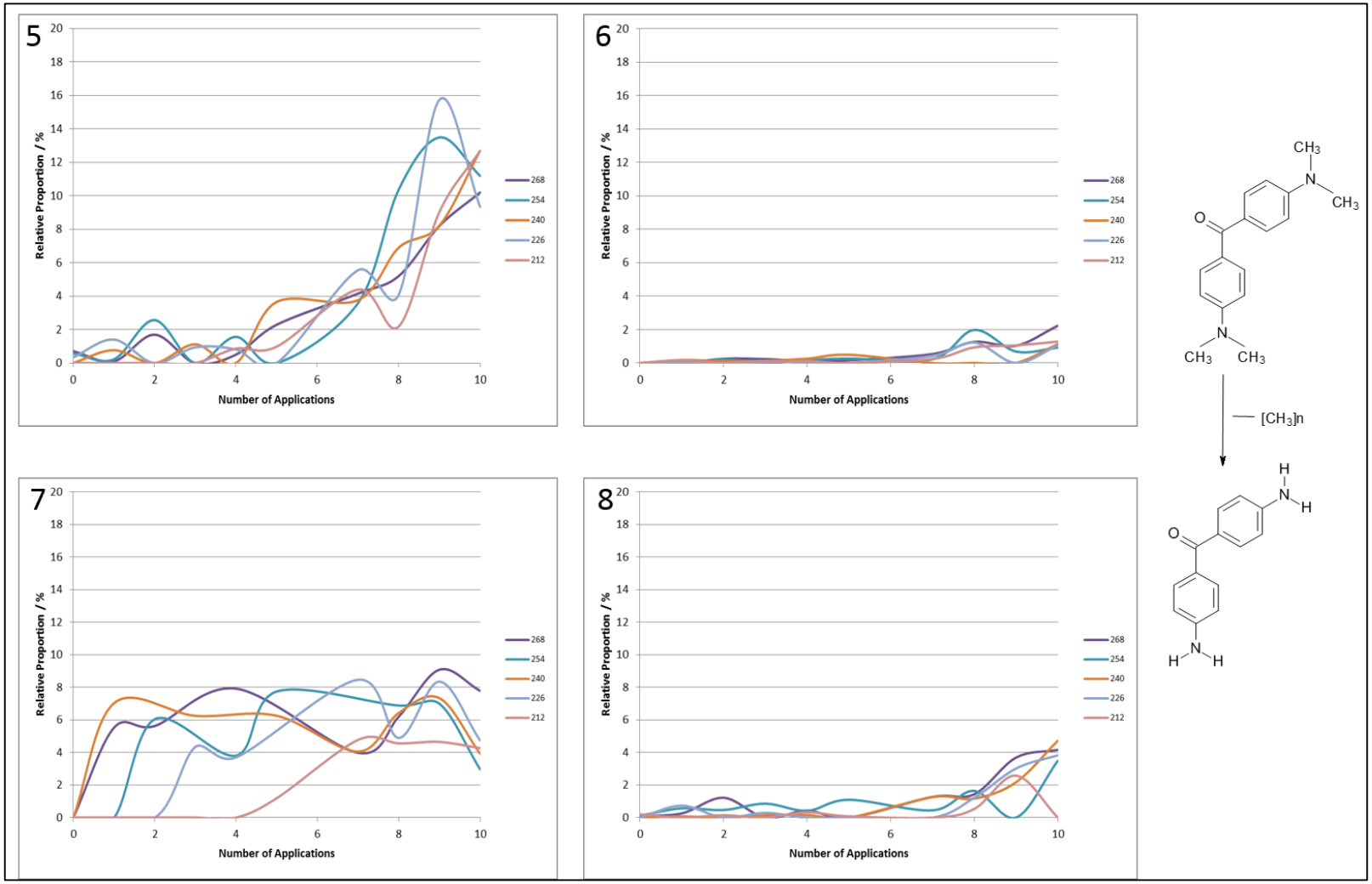


Figure 5.21 Aging Curves for Benzophenone Products for Basic Violet 3 in Black Pens 5: Micron, 6: Bic, 7: Staedtler, 8: Woolworths after treatment with hydrogen peroxide

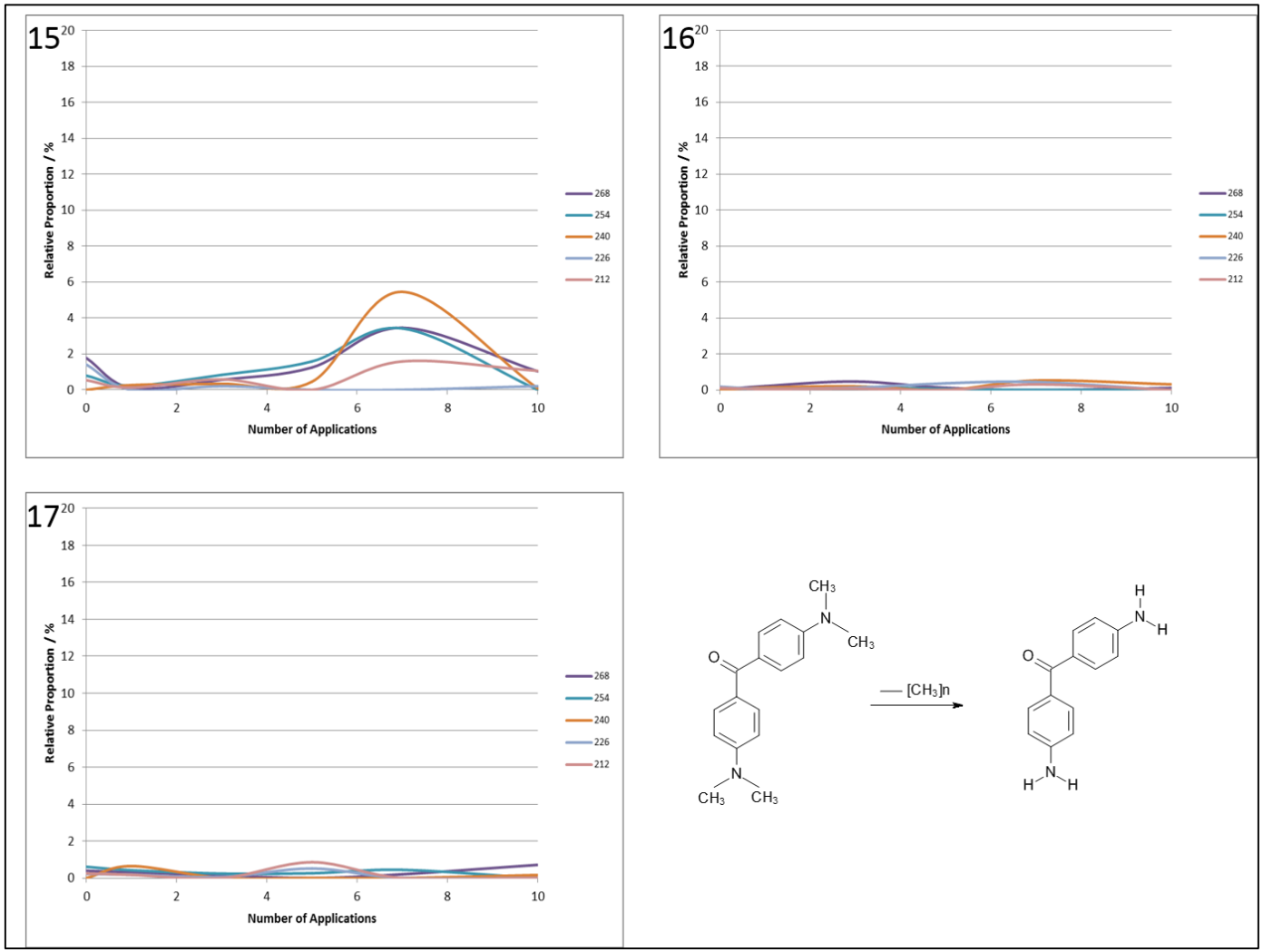


Figure 5.22 Aging Curves for Benzophenone Products in Basic Violet 3 in black Pens 15: Papermate, 16: Galileo, 17: Papermate after treatment with hydrogen peroxide

These results have shown examples of how hydrogen peroxide has influenced the fading of the inks but that even though inks may contain the same dyes, the degree of degradation was not consistent between samples.

5.4 Bleaching with Sodium Hypochlorite

5.4.1 Bleaching of Dye Standards

Each dye standard had 2 µl of 5% household bleach added to it before being monitored by APCI-MS. Samples were kept in the dark or subjected to artificial daylight over a period of time to initiate the degradation process of bleaching. Analysis was carried out immediately after treatment then after 2, 17, 28 and 40 days.

5.4.1.1 In the Dark

The standard dye solutions were stored in a cupboard during this stage of analysis in order to be able to compare with samples subjected to artificial daylight. The mass spectra for dyes Basic Violet 3 and 1 are shown in Figures 5.23 and 5.24.

Figure 5.23 shows the mass spectra for Basic Violet 3 after treatment with 5% bleach. As expected, the peak for the molecular ion m/z 372 was most abundant with m/z 358 (mono-N-demethylated) slightly smaller. By 17 days, m/z 358 was more prominent than m/z 372 and remained so for the whole time period. Also at this time the other demethylation products m/z 344 and 330 started to increase. At 40 days all previous components were still present with m/z 358 the highest intensity. Here m/z 330 had increased and was greater than 344. There was evidence of benzophenones present, as there was a peak visible at m/z 268 which corresponds to di-methyl, di-methylaminobenzophenone. These results show the degradation of Basic Violet 3 by bleach over time by demethylation and breakage of the central bond.

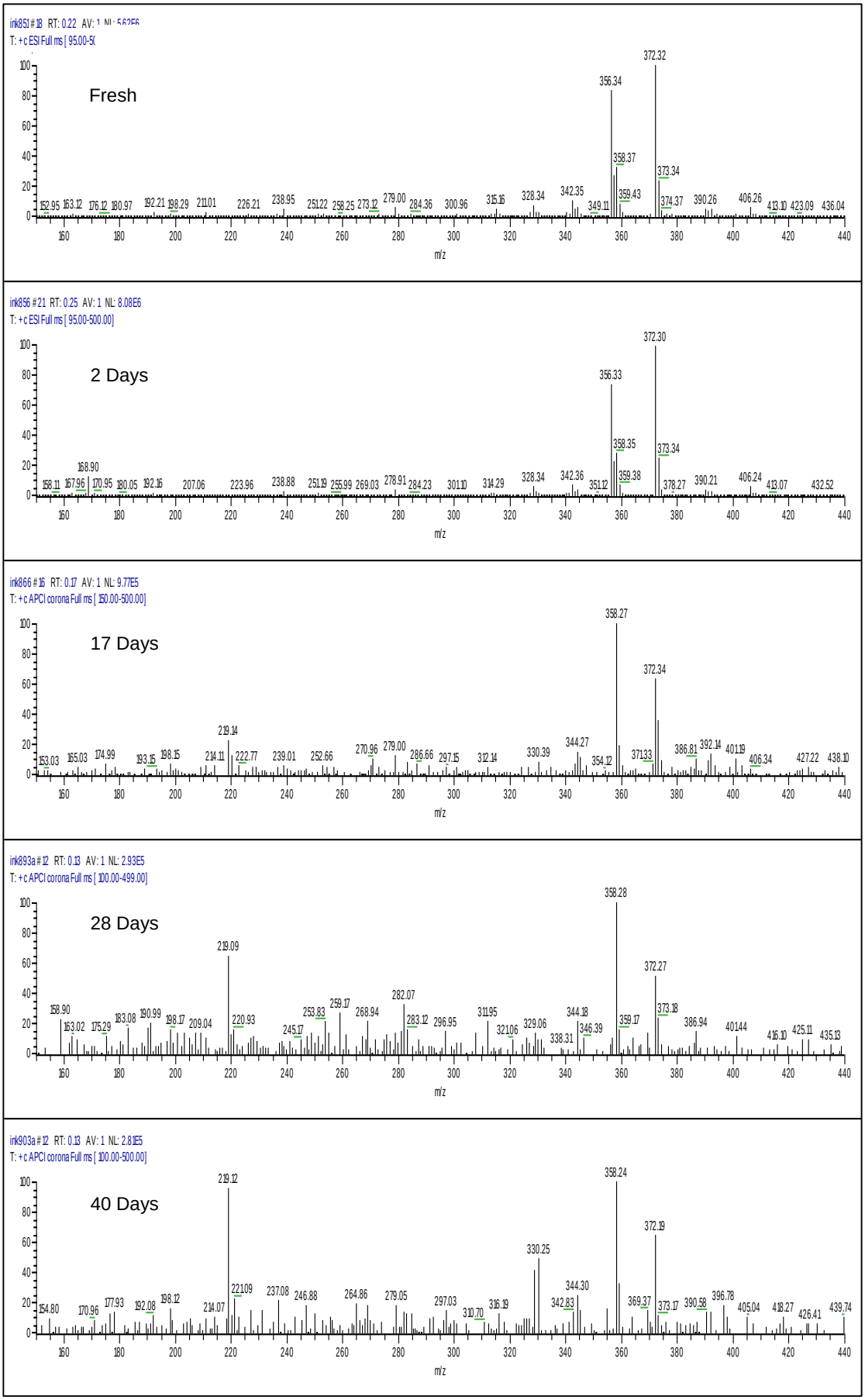


Figure 5.23 APCI-MS Spectra for Basic Violet 3 during Treatment with 5% Bleach

Basic Violet 1 also degraded with bleach as illustrated in Figure 5.24. Peaks at m/z 358, 372, 344 and 330 were all clear to see in the fresh sample, with m/z 358 as expected, the most abundant, followed by m/z 372. After 2 days there were few visible differences, however by 17 days, all the dye peaks were significantly smaller, which was attributed to the contaminant peak at m/z 270; however they can still be identified and m/z 358 was still the most abundant. By 40 days, the dye peaks were not clearly visible so the actual MS data was needed to determine if they were still present. Basic Violet 1 appeared to follow the same demethylation process of degradation as Basic Violet 3. The peak visible at m/z 219 was not a result of degradation, but from contamination within the equipment.

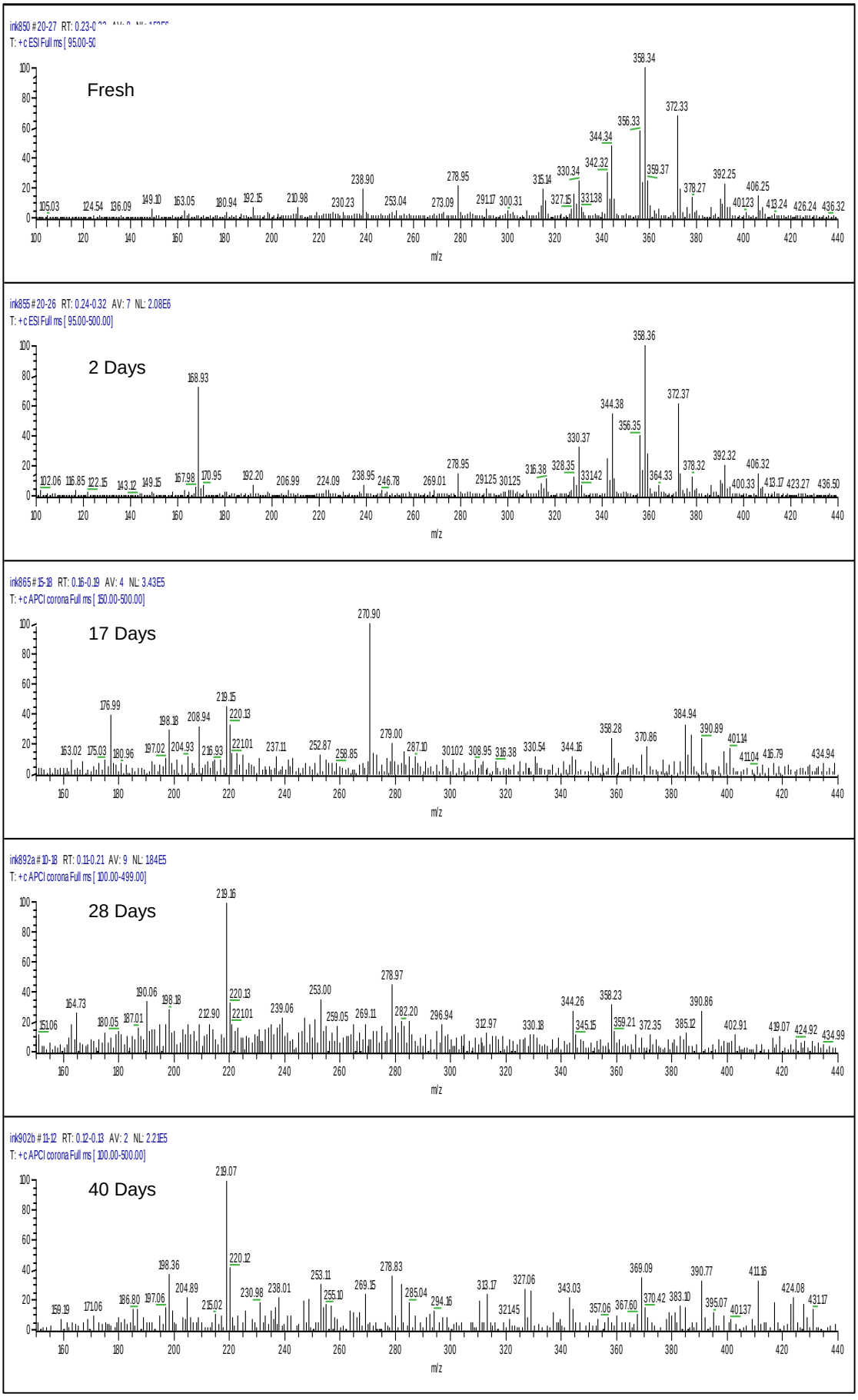


Figure 5.24 APCI-MS Spectra for Basic Violet 1 during Treatment with 5% Bleach

Using the data from the mass spectra, aging curves were created and Figure 5.25 shows the changes that occurred for Basic Violet 3 and 1 over the 40 days.

As seen in the mass spectra and as shown in the aging curve, both parent dyes decreased in RP over the 40 days, and the homologues increased suggesting degradation was taking place due to bleach. This was supported by the ion count data. For Basic Violet 3 at T = 0 m/z 372 was 100% and dropped to 42% at T = 40 days and for Basic Violet 1 m/z 358 dropped from 100% to 6%. This also supports previous evidence that more degradation has occurred for Basic Violet 1 than Basic Violet 3.

There were still coloured components detectable and visible after 40 days, as the bleach had not completely decolourised the Basic Violet 3 solution.

As well as demethylation and deethylation, cleavage of the central phenyl bond can occur which produces benzophenone products. Graphs c and d in Figure 5.25 show the aging curves for these products for Basic Violet 3 and 1 after treatment with 5% bleach. The RP's were not particularly high, none were above 4% for Basic Violet 3, but it does indicate that benzophenone products were produced, as the proportions increased over the 40 days. For basic Violet 1, the RP values were higher for all products supporting the theory that more degradation takes place for this dye than Basic Violet 3.

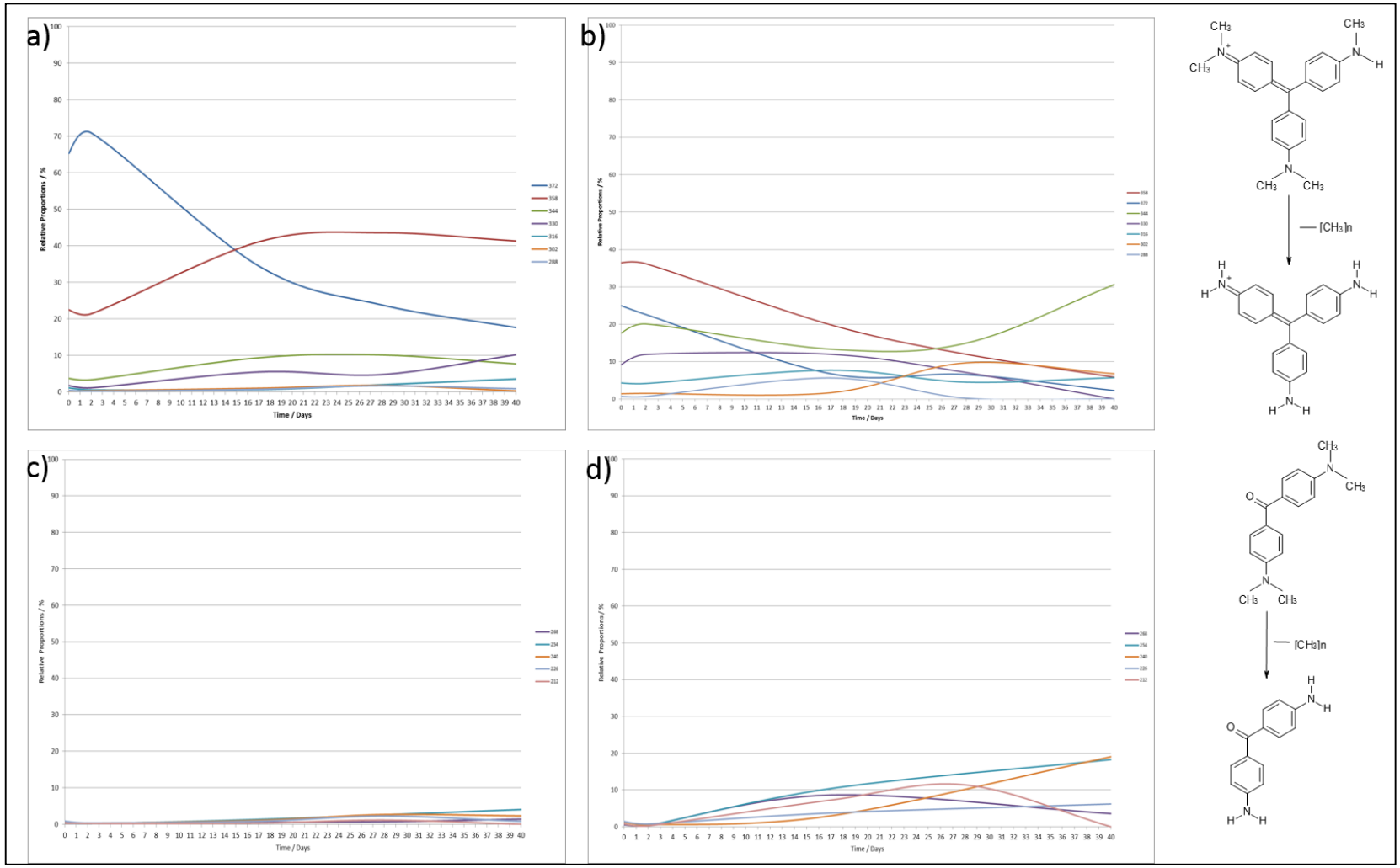


Figure 5.25 Aging Curves for dye standards a: Basic Violet 3, b: Basic Violet 1, c: Benzophenones for BV3, d: Benzophenones for BV1 during treatment with 5% bleach

Figure 5.26 displays the aging curves for both dye components. It can be clearly seen that Basic Violet 1 produced benzophenone degradation products more quickly than Basic Violet 3 indicating that Basic Violet 3 was more stable than Basic Violet 1. These results are consistent with previous results from photofading and peroxide bleaching.

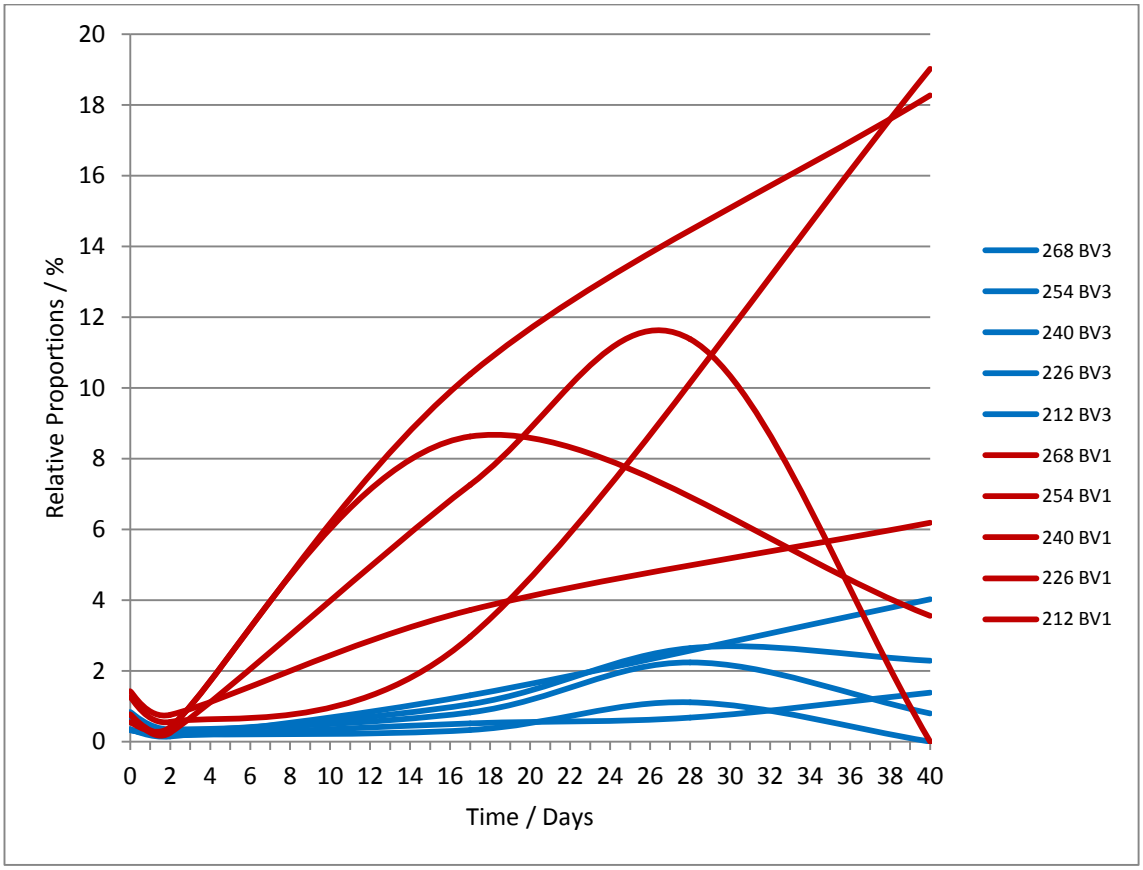


Figure 5.26 Aging Curves for Benzophenone Products for Basic Violet 3 and Basic Violet 1 during Treatment with 5% Bleach

Phenol products can also be produced due to the breaking of the central bond. For dyes Basic Violet 1 and 3 the RP values were not particularly high, but relative ion count data supports that m/z 137 increased from 0 to 4% for Basic Violet 3 and 0-12% for Basic Violet 1.

Table 5.12 shows the relative proportions of the component types when fresh and after 40 days. It shows a reduction in dye components for Basic Violet 3 and 1, with the degradation greater for Basic Violet 1 as it reduced from 94% to 51%, but Basic Violet 3 only reduced by 10%. There was an increase in the benzophenone degradation products, but it was significant for Basic Violet 1 increasing from 5 to 47%. This corroborates the results of increased degradation

for Basic Violet 1. Phenol products did increase for Basic Violet 3 and 1, but only by 2%.

Table 5.12 Table to show the Relative Proportions of Products based on Ion Count Data after Treatment with 5% Bleach

	Basic Violet 3		Basic Violet 1		Basic Blue 26	
	T=0	**T=40**	**T=0**	**T=40**	**T=0**	**T=40**
Dye /%	98	89	94	51	100	99
Benzophenones /%	2	8.5	5	47	0	1
Phenols /%	0	2.5	0	2	0	0

These results have shown that even a small amount of bleach can have an effect on the dyes but the effects are not always the same for all of the dyes.

5.4.1.2 In the Light

In addition to the use of household bleach to treat the dye standards, they were also subjected to artificial daylight in combination with bleach to see what affect it would have on the dyes and the degradation process. The mass spectra for the black dyes can be seen in Figures 5.27 and 5.28.

Figure 5.27 clearly shows how the main peaks at m/z 372 and 358 became less abundant and peaks m/z 316 and 288 increased over time. When light was used in combination with bleach, Basic Violet 3 degraded faster than without light. After 5 days, the peaks corresponding to m/z 372 and 358 had significantly reduced in intensity and the known degradation products were being seen. Peaks at m/z 330, 316 and 302 represent the tri, di and mono-methylated homologues for Basic Violet 3 with a mass difference of 14. At 9 days, the final homologue (hexa-demethylated) m/z 288 was the predominant peak present in the spectra, with all methyl groups lost from the original structure. The other homologues were present but in much smaller quantities.

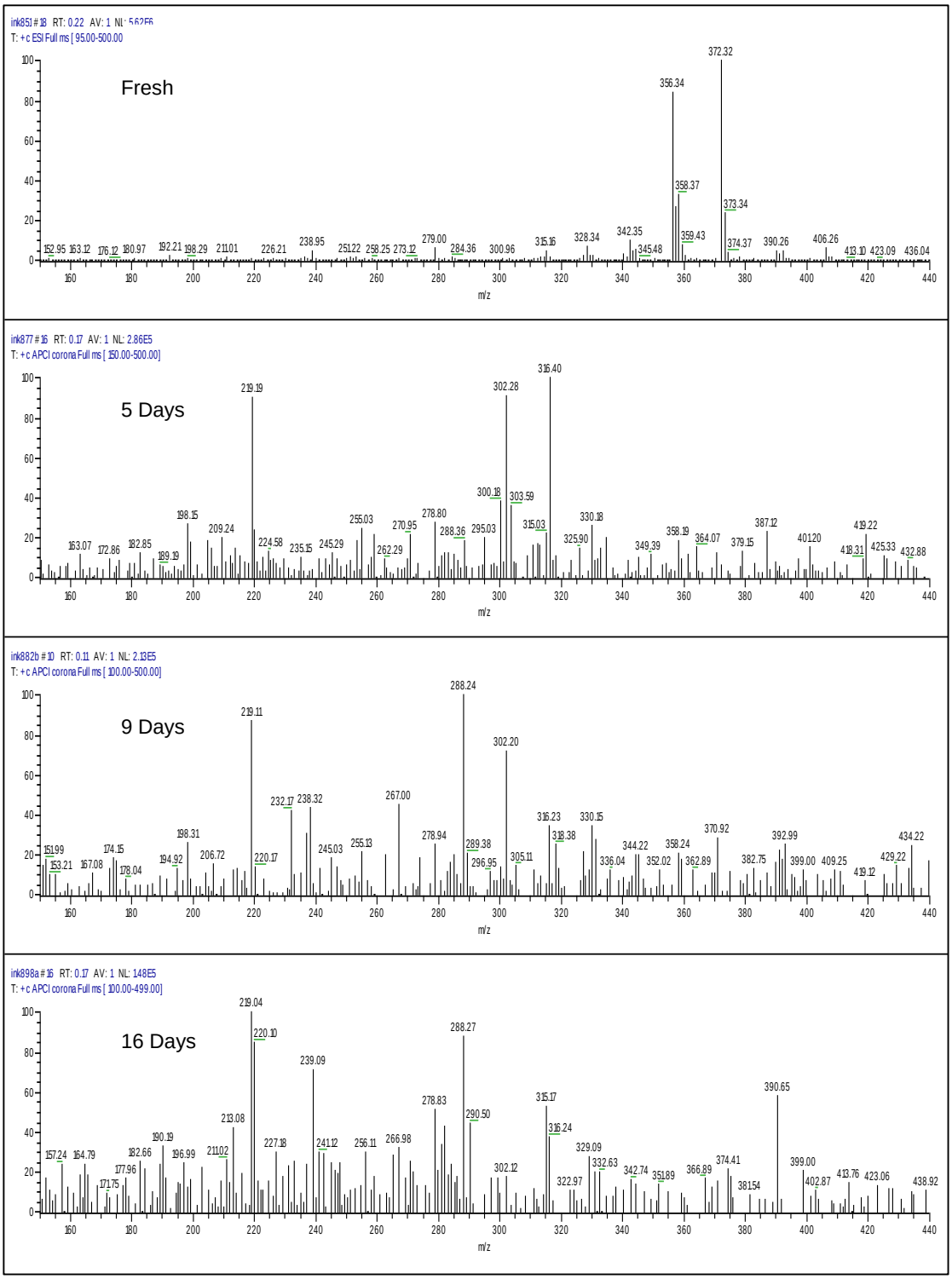


Figure 5.27 APCI-MS Spectra for Basic Violet 3 during Treatment with 5% Bleach and Light

Basic Violet 1 responded to the bleach and light in a similar way that Basic Violet 3 did as shown in Figure 5.28. After 5 days, the predominant peak was m/z 330, (di-demethylated) with 358 and 372 considerably minor in comparison. Peaks at m/z 316 and 302 were present, but unlike Basic Violet 3 m/z 288 was not the main degradation homologue.

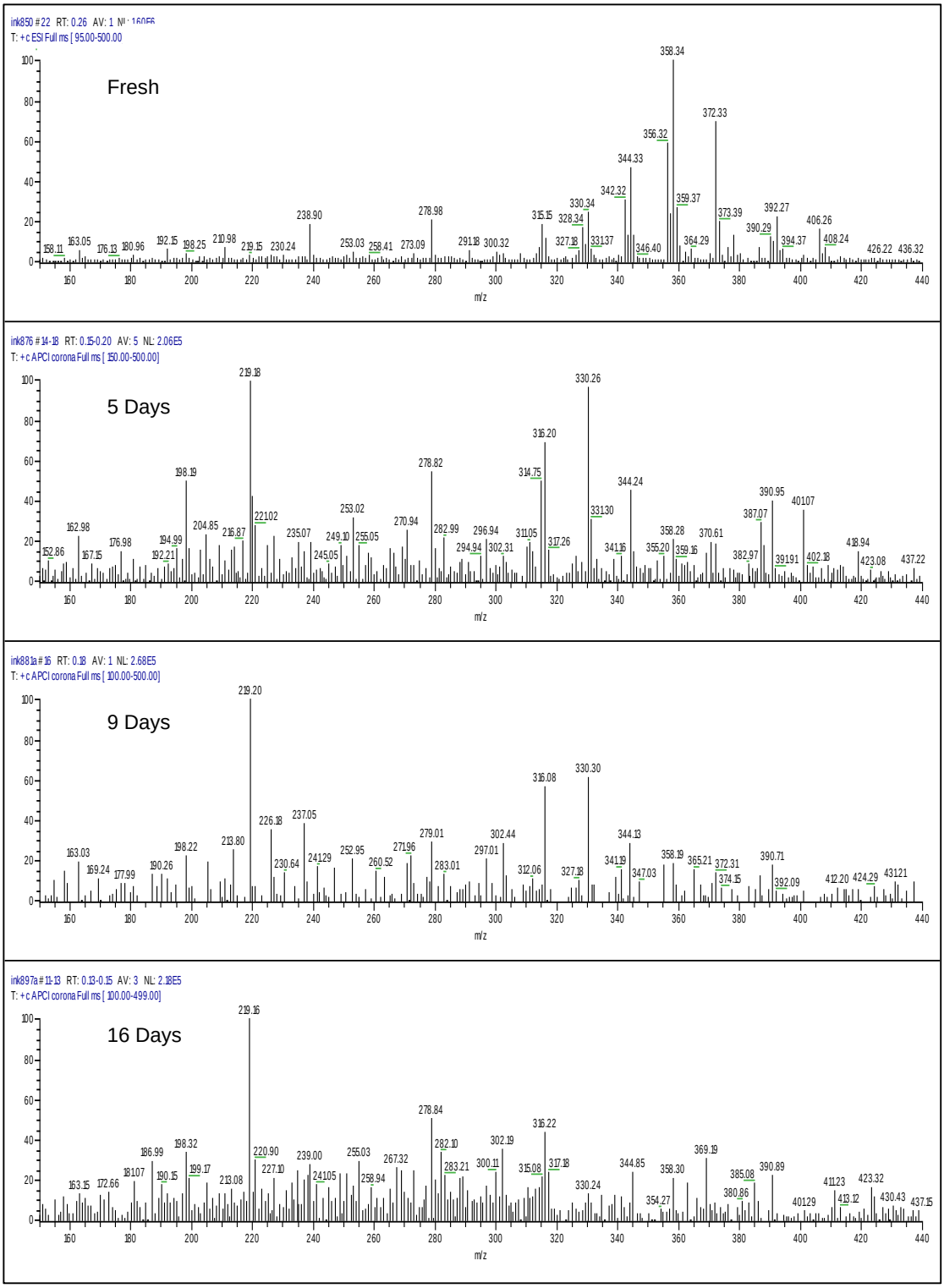


Figure 5.28 APCI-MS Spectra for Basic Violet 1 during Treatment with 5% Bleach and Light

Figure 5.29 displays the aging curves for Basic Violet 3 and 1 following treatment with bleach and light.

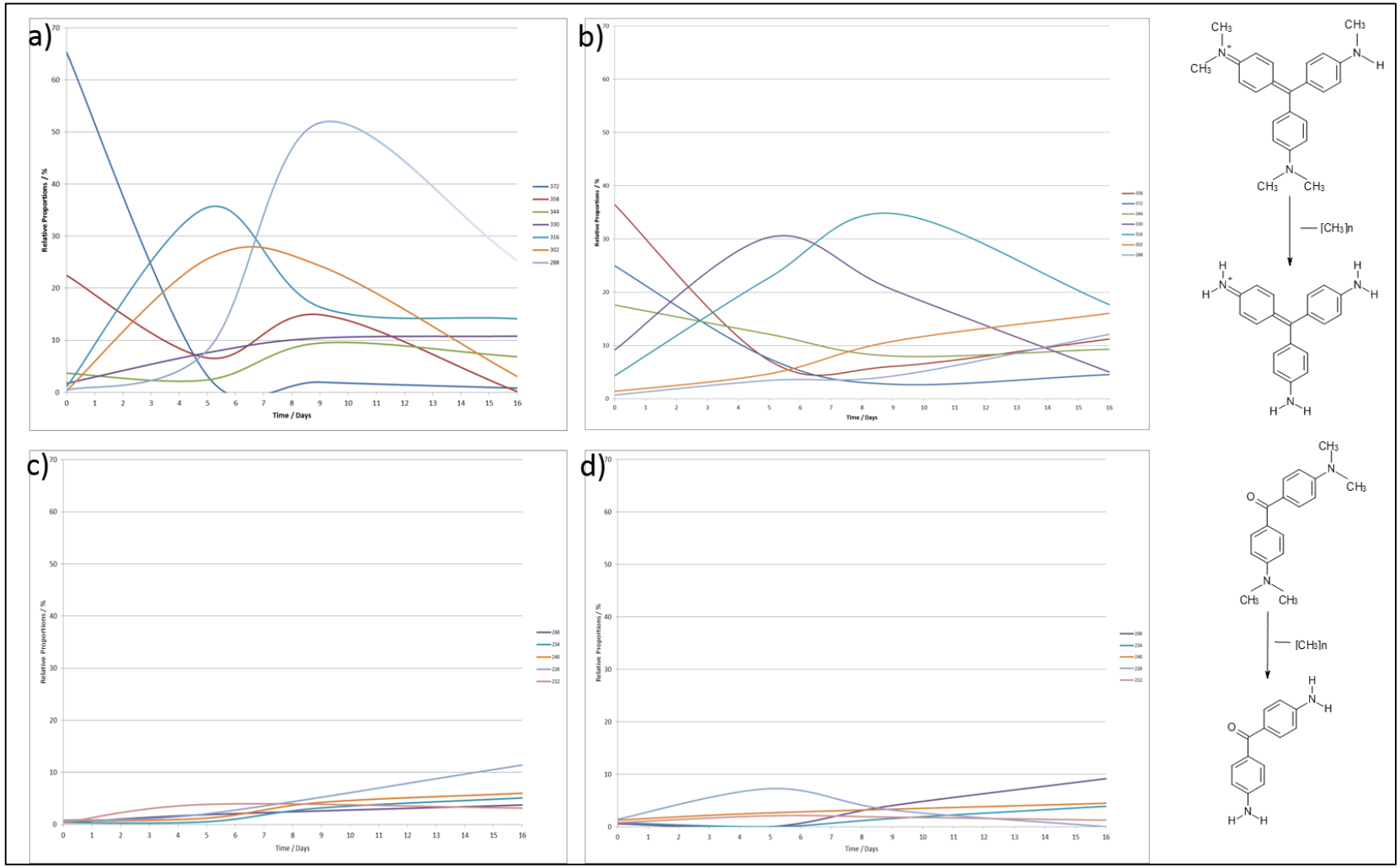


Figure 5.29 Aging Curves for dye standards a: Basic Violet 3, b: Basic Violet 1, c: Benzophenones for BV3, d: Benzophenones for BV1 during treatment with 5% bleach and light

With bleach and light there was a lot of activity with Basic Violet 3 compared to without light as shown in graph a) in Figure 5.29. All degradation homologues showed an increase in their relative proportions almost immediately which could correspond to the quick degradation of m/z 372 and the production of its products via demethylation. Relative ion count data supports the RP data. At T = 0, m/z 372 was 100% and dropped to 1% by T = 16 days. The most abundant component at 16 days was m/z 288 (0 Me) with a relative ion count of 37%. In comparison, the homologues for m/z 316 (2 Me) and m/z 330 (3 Me) were most prominent with Basic Violet 1. The relative ion data showed that m/z 358 decreased from 100% at T = 0 to 24% at T = 16 days and the most abundant component at 16 days was m/z 316 (tri-demethylated) which had increased from 12% – 38%. This data is different to all other data so far with regards to the degradation of these two dyes. Under all other conditions, Basic Violet 1 appears to go through more degradation than Basic Violet 3, whereas this condition of bleach and light would indicate more degradation for Basic Violet 3.

Table 5.13 shows the relative proportions of the component types when fresh and after 16 days based on ion count data. As seen previously for photofading and peroxide treatment the dye components have reduced in amount and the degradation products have increased. Differently for bleach, Basic Violet 3 appears to have had more degradation with light than Basic Violet 1 and compared to all other treatments. Phenol products have increased for all dyes, unlike without light.

Table 5.13 Table to show the Relative Proportions of Products based on Ion Count Data after Treatment with 5% Bleach and Light

	Basic Violet 3		**Basic Violet 1**	
	T=0	**T=16**	**T=0**	**T=16**
Dye /%	98	65	94	75
Benzophenones /%	2	29	5	20
Phenols /%	0	6	0	5

Figure 5.30 shows the comparable aging curves for the blue dye standards. It can be clearly seen that light has had an effect on their degradation compared to just bleach treatment whereby the degree of degradation has increased.

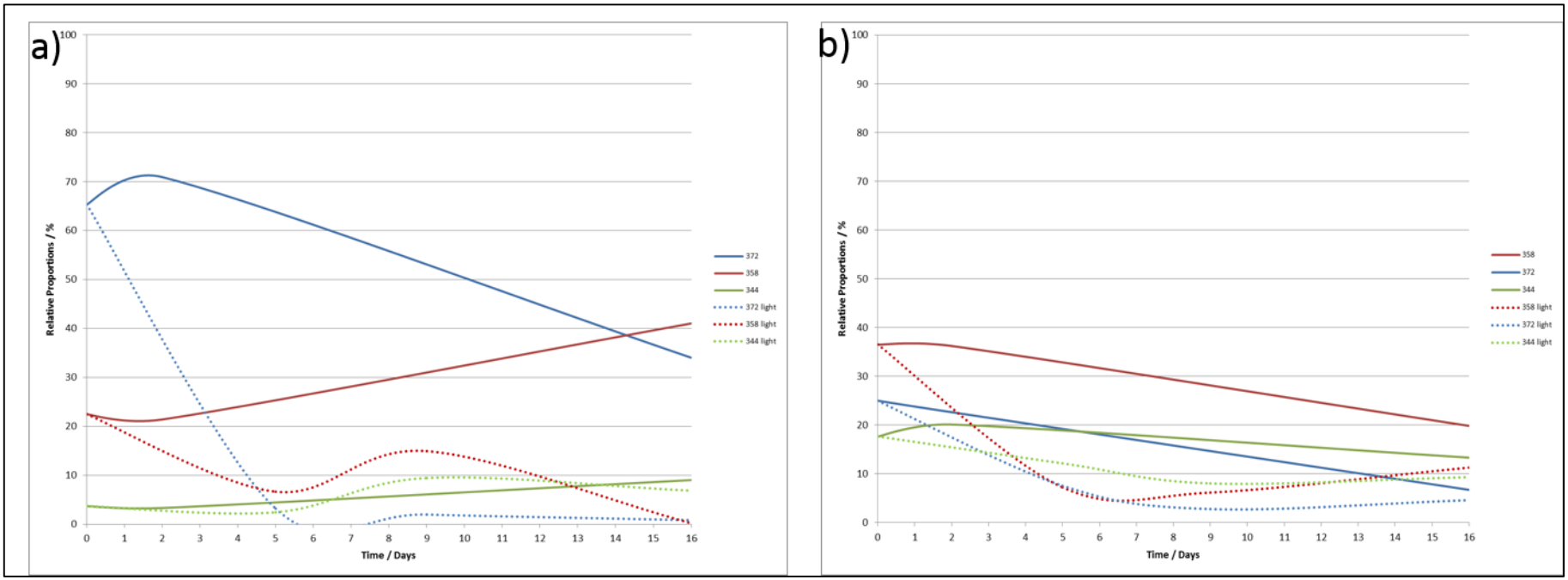


Figure 5.30 Aging Curves dye standards a: Basic Violet 3, b: Basic Violet 1, during Treatment with 5% Bleach with and without Light

5.4.2 Bleaching of Inks on Paper

As explained in Chapter 2, bleach was applied to the ink samples when written on the paper surface. Figure 5.31 shows the visible light results after ten applications of household bleach to all samples.

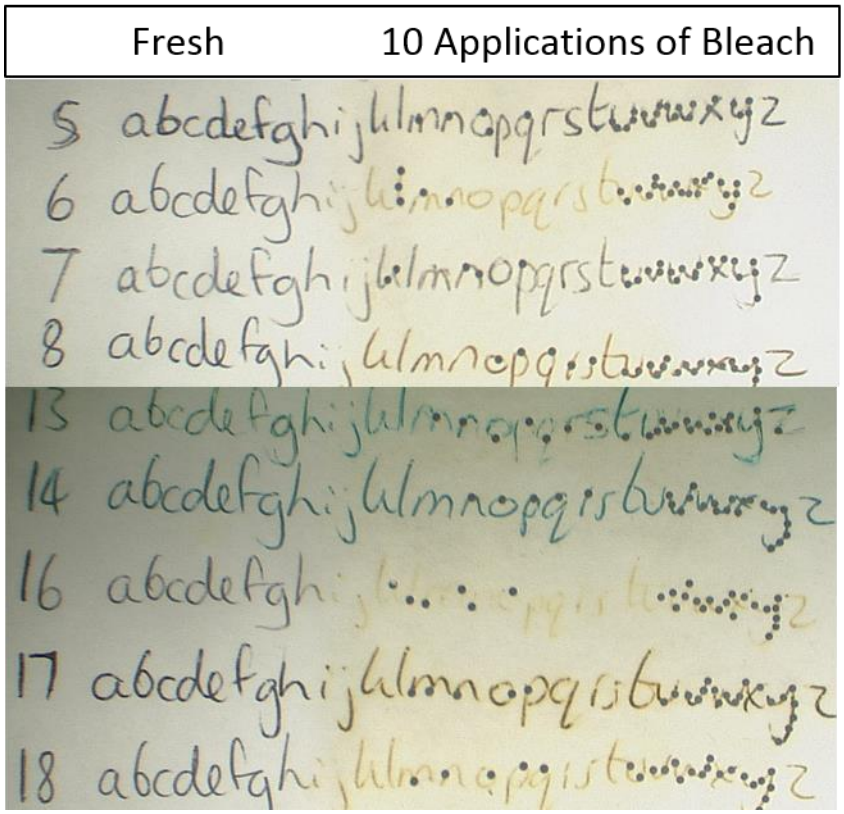


Figure 5.31 VSC Visible Light Image of Fresh Black Ink and Ink treated with 10 applications of 5% Bleach

After only one application of bleach, the inks were slightly lighter than without, but no significant fading had occurred. After ten applications fading had occurred for all samples, but not to the same extent as seen in Figure 5.31. Pen 16 (Papermate Flexi) showed the most significant fading, with little colour remaining. Pens 5 and 7 still exhibited a black colour but pens 6, 8, 16 and 18 were now a brown or yellow colour. There did not appear to be any affect from the bleach to the immediate vicinity of the ink line like with some of the peroxide samples, but the paper generally did become yellow if subjected to bleach.

Fluorescence was also examined for the bleach samples and the results are shown in Figure 5.32.

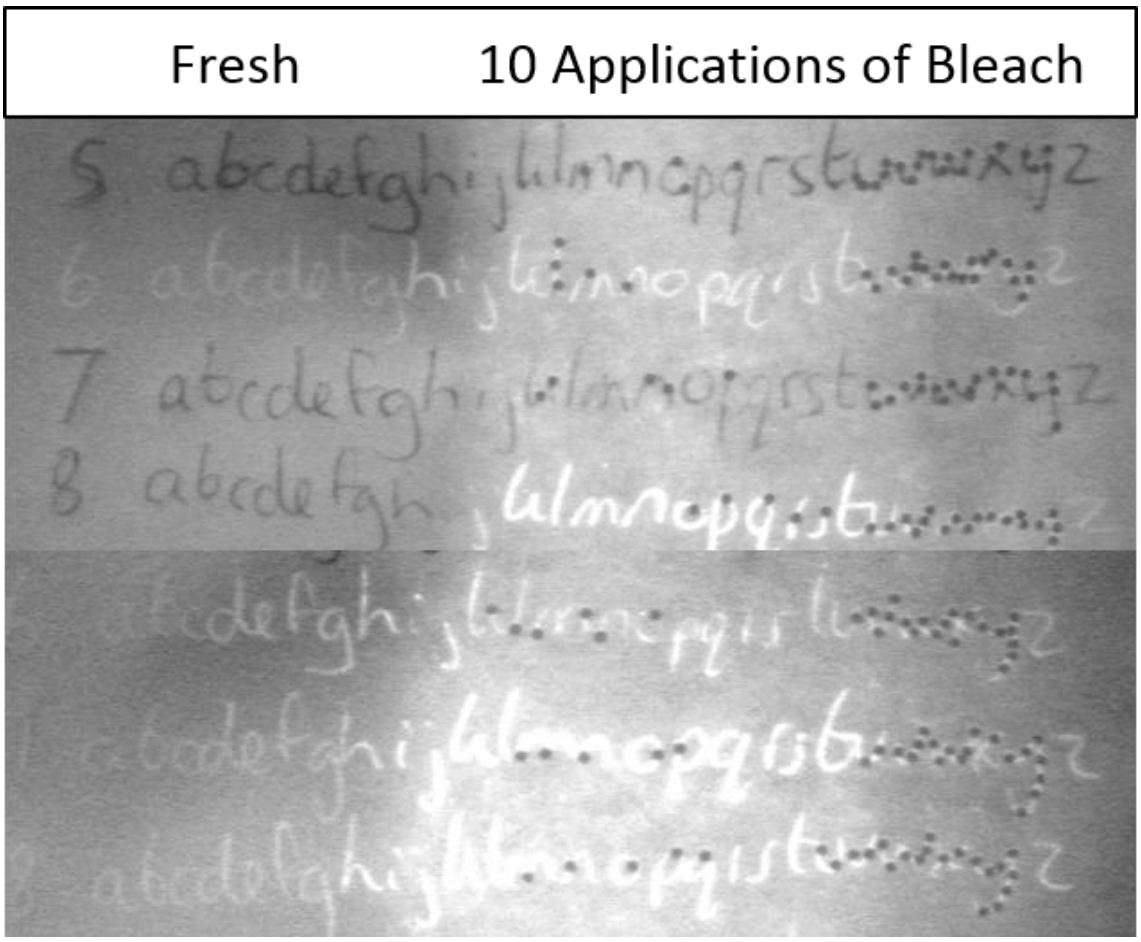


Figure 5.32 VSC Fluorescence of Black Pen Samples Fresh and after 10 applications of 5% Bleach

Pens 6, 16, 17 and 18 all exhibited fluorescence with and without bleach treatment, but fluorescence appeared enhanced for pens 17 and 18. Pen 8 did not fluoresce pre-treatment but fluorescence was induced by bleach treatment. It was also clear to see the area in which the bleach had been applied to the paper (letters j-z) as the paper was brighter. Pen 8 contained Basic Blue 9 which as explained previously fluoresces when colourless which could explain the results for this pen, but there are clearly different components in all the pens which are giving the results as one common component has not been identified, but even then not all of the samples fluoresce. More investigation is needed on this topic to determine the exact reasons for the fluorescence.

These results were similar to results from photofading and peroxide bleaching in that fluorescence enabled the re-visualisation of faded ink, but different samples fluoresced under different conditions.

APCI-MS data was analysed to determine the aging curves of the pen inks on paper post bleach treatment as shown in Figures 5.33 and 5.34 for the dye Basic Violet 3 present in all the black pens. Visually the Papermate ink (15) and Bic (6) were the most faded and Micron (5) was the least faded, however this was not fully supported by the aging curves and the ion count data. For most of the pens, m/z 372 had a consistent proportion during the treatment indicating little degradation. However for pens 5, 7 and 15 the RP values decreased but also the ion count data reduced from 100% at T = 0 to less than 10% at T = 10. For the other pens, the values did not decrease by the same amounts. This would suggest greater degradation for pens 5, 7 and 15 compared to the other black pens. This result was different to the visual observation in which Micron (5) did not show signs of colour fading and Bic (6) did. For the majority of the pens, the aging curves did not display any significant aging, but there were differences between all of the pens. This information has shown that a visual observation cannot be relied upon to determine the degree of fading when samples have been treated with bleach. It also shows that bleach does not influence degradation as much as peroxide bleaching, but inks can be discriminated between based on their aging curves.

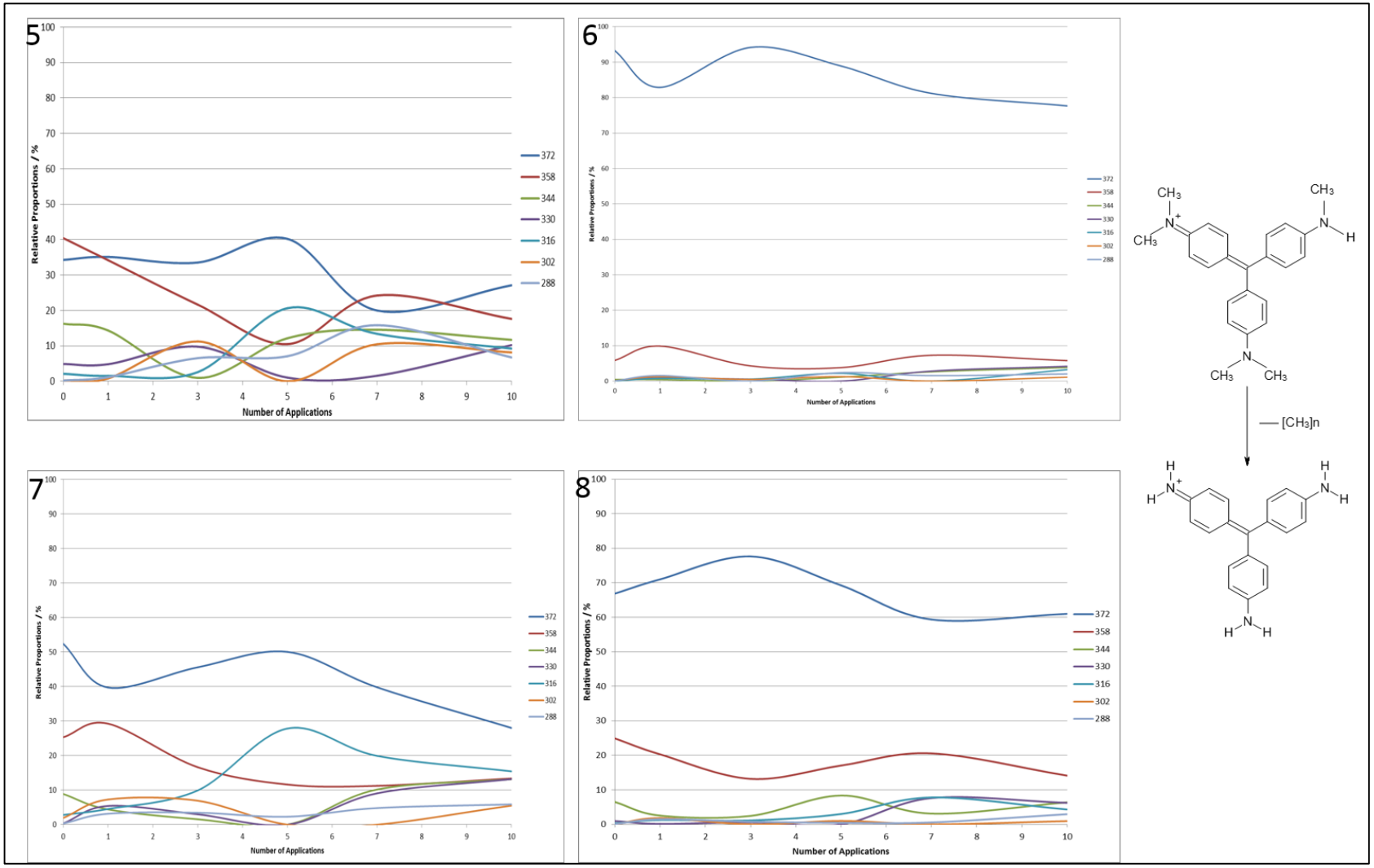


Figure 5.33 Aging Curves for Basic Violet 3 in Black Pens 5: Micron, 6: Bic, 7: Staedtler, 8: Woolworths after treatment with 5% bleach

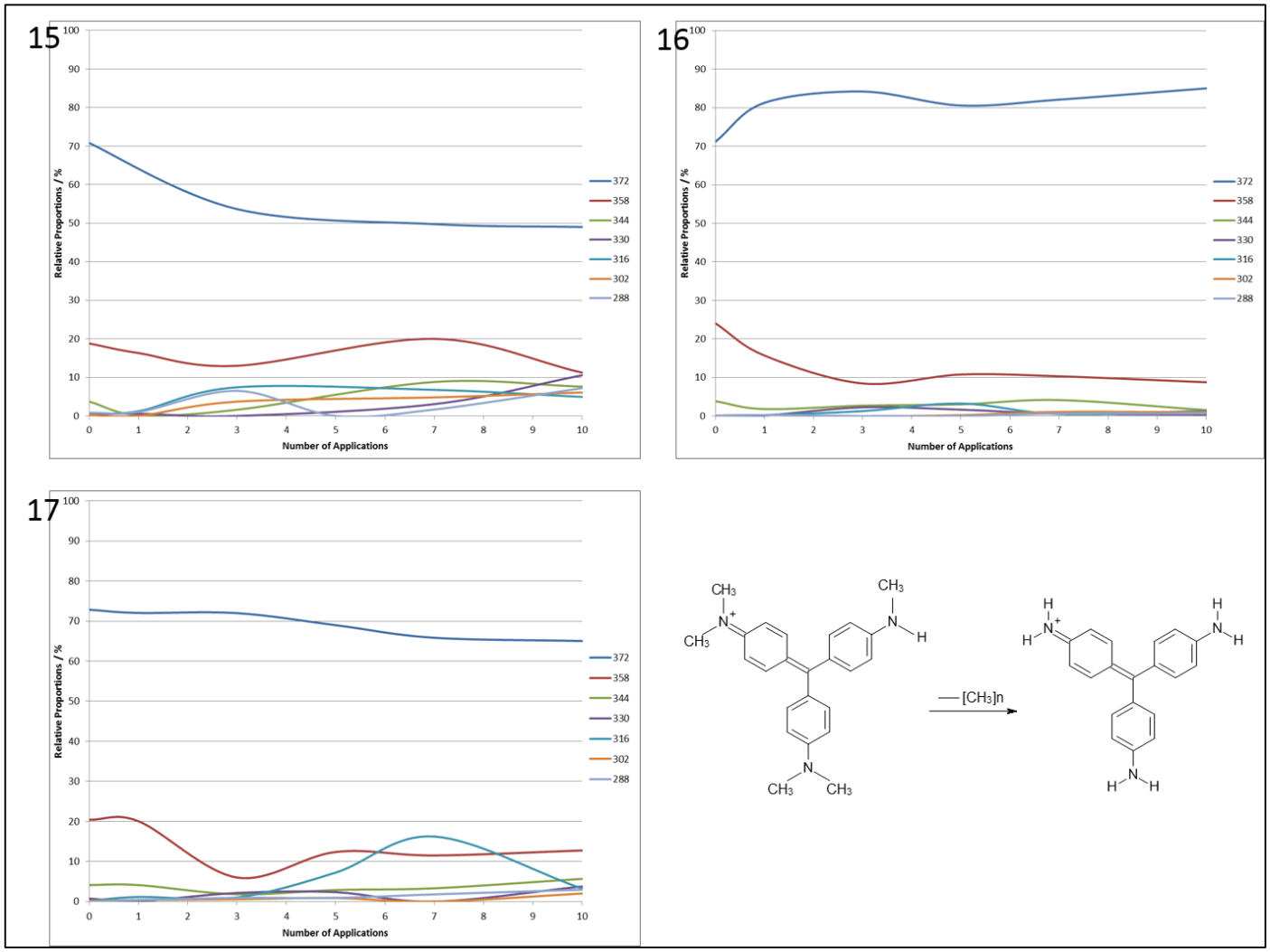


Figure 5.34 Aging Curves for Basic Violet 3 in Black inks 15: Papermate, 16: Galileo, 17: Papermate after treatment with 5% bleach

5.5 Discussion and Conclusions

5.5.1 Photofading

When the samples were exposed to light, the number of degradation products and their quantities increased with time which was consistent with previous research.[18] Furthermore the aging curves clearly confirm a successive production of the products, which was also consistent with the literature.[154] Their RP values would increase and decrease at different times in their order of degradation, e.g. for Basic Violet 3 m/z 316 reached its peak at 5 days, m/z 302 at 7 days and m/z 288 at 9 days. Basic Violet 1 also showed the succession, but at 5 days m/z 330 was greatest and at 9 days m/z 316. Degradation was clearly enhanced with light, but the rates for all dyes were still different. Relative Proportion and aging curves enable us to see the stability of a dye and its components and how they change over time, but they cannot tell us the rate of degradation. Relative ion count data however, can give information about the degree of degradation and in support of the results from RP, it showed that Basic Violet 1 had a greater degree of degradation that Basic Violet 3.

For the dye mixtures, there did not appear to be any successive degradation as seen with the dye standards alone, but the later homologues; m/z 288 and 316 were common in the mixtures. Mixture 4 appeared to exhibit the greatest degradation which corresponds to pen 5 (Micron), but this was in fact the most stable of the black inks on paper. These results show that the dyes behave differently when in solution and when on a substrate.

The ballpoint ink samples showed the sequential loss of methyl and ethyl groups, but also the successive production and degradation of products, shown as fluctuating peaks in the aging curves. This however was only present when subjected to external factors such as variable light flux. Dyes degrade strongly under the influence of light, but very slowly or not at all in the dark. Samples stored in the dark in a folder, did not exhibit any significant signs of degradation over the same time period which was consistent with previous studies.[18] If degradation does occur in the dark, further research is required to confirm if the pathways of degradation are via *N*-demethylation or hydration.

Degradation products increased with time exposed to light, but they did not increase continually. They appear to level off after longer exposure times, implying that the degradation process reaches a limit or is slowing down significantly. This can be explained because as a dye degrades via demethylation the wavelengths at which it will absorb are shifting to shorter wavelengths (hypsochromic), for example λ_{max} for Basic Violet 3 that has six methyl groups is 590 nm, but for pararosaniline the homologue that has no methyl groups, λ_{max} is 544 nm. Figure 5.35 shows the light intensity vs wavelength for the solar spectrum which was replicated by the artificial daylight bulbs.

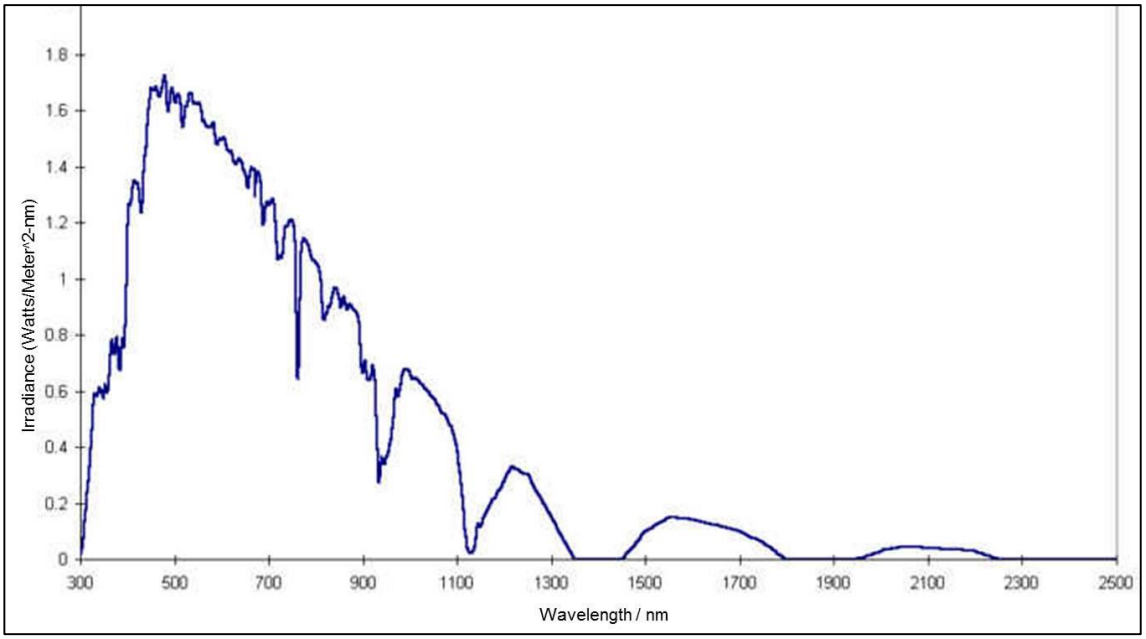


Figure 5.35 Typcial Solar Spectrum

It can be seen that as the wavelength of light decreases, the intensity increases till approximately 500 nm at which point there is a sudden decrease to 300 nm. As the dyes degrade through the wavelengths and the λ_{max} decreases, the molar absorbance decreases so less light will be absorbed by the molecules, giving lower yields of singlet oxygen and therefore lower yields of degradation products. Also, the width of a typical absorption band peak extends approximately +50 nm to -150 nm from the λ_{max}, so the majority of the absorption band for demethylated dyes will fall progressively further into the <500 nm drop off region, which means less reaction with progressive demethylation. The benzophenone products do fall into the low intensity <500 nm region and so should be stable to daylight, but not to dye generated singlet oxygen, so if there are still dye components present the benzophenones will demethylate.

From analysis of all APCI mass spectra data for photofading it is believed that benzophenone products were present in the ink samples tested and also phenols, however they were present in small quantities.

Results so far support the literature in that light influences the degradation process, but in real situations photofading could occur naturally from sunlight, or deliberately using artificial light sources. New findings concern the use of luminescence to enhance faded entries caused by photofading. Fluorescence was observed in samples after photofading that were colourless, which made the writing visible. Further investigation is needed to determine the exact component causing the fluorescence in the inks.

Other oxidation processes may also be used to affect the fading process; for example hydrogen peroxide can be obtained quite easily and is present in some household products which could be used to alter information written on documents deliberately.

5.5.2 Bleaching with Hydrogen Peroxide

When the samples were treated with hydrogen peroxide, the number of degradation products increased. With 2 µl of 5% H_2O_2, all dye standards had visibly faded and Basic Violet 3, Basic Violet 1 were colourless by 8 days. The successive degradation and subsequent production of homologues seen with photofading were not apparent when peroxide was used. There does not appear to be an order in which products were formed. Degradation was occurring however from peroxide and the lower homologues e.g. m/z 302 and 288 for Basic Violet 3 and 1 were the dominant products at the end of the analyses. Benzophenone products were also present with m/z 254 and 226 prominent for both dyes and the RP values between 15 – 35%. Phenol compounds were detected in differing amounts for the different dyes for example Basic Violet 3 had values of 6%, but for Basic Violet 1 all values were below 2%. There is limited information about reactions between hydrogen peroxide and dyes in the absence of light. Some studies have said that decolourisation does not occur when only H_2O_2 is used,[157] another stated that a small amount of colour loss does occur by

H_2O_2 alone.[153] These papers were interested in dyes for environmental reasons and the conditions of the research were different to those used in this thesis, for example, different dyes and different techniques used to analyse the photochemical oxidation of the dyes, so the exact results will not be the same.

When light was used in combination with peroxide, degradation occurred as would be expected, and it appeared that light increased the degradation, however there was still a lack of successive degradation as seen previously. Benzophenone and phenol products were again produced, but with different values to when only peroxide was used.

A difference was observed between photofading and bleaching for the mixtures. In light, the later homologues (m/z 288, 316) would be present and have the highest RP values in the aging curves, but in peroxide bleaching, the early homologues (m/z 344, 330), would be present with the highest values. This would indicate that the degradation process was occurring in a different way under the two conditions; in light, the process may be successive and the homologues were generally formed one after the other, whereas in peroxide, the degradation could be quicker and the homologues were not produced at a rate that was quicker than they were destroyed, which would actually produce more of the later homologues. Benzophenone products were detected and their RP values were largely higher from peroxide bleaching than photofading. This could be explained because hydroxyl radicals (which can be formed from hydrogen peroxide) have been recorded as attacking the chromophore structure leading to the generation of benzophenone and phenol products.[158] Also when light and peroxide are used in combination, singlet oxygen would be produced by some dyes due to the light and hydroxyl radicals produced by the peroxide which would mean more reactions would be happening and consequently the rate of degradation would be faster. If using the ion count data, the greatest degradation in the mixtures occurred for mixture 4 once again which corresponded to ink 5 (Micron) which was not the most faded of the inks on paper, that was ink 7 (Staedtler). The dyes have once again behaved differently on paper than in solution.

On paper, visible results were seen from peroxide bleaching of the ink, but the fading of the inks was not uniform, showing that the rates of degradation for

different inks and therefore different dyes was not the same. It also highlighted how some inks were more resistant to attack than others. An interesting observation from the VSC 5000 fluorescence results was the decrease in fluorescence for one of the black inks following peroxide bleaching. This was unusual as for the other samples where fluorescence was present it was enhanced. A study did observe this for the pure dye Basic Red 1 in solution which was fluorescent, but following treatment from a strong oxidiser such as hydrogen peroxide it caused the fluorescence to disappear.[159] Fluorescence was induced for one sample by the peroxide treatment, but no explanation is available to date as to why. The fluorescence was useful however for a number of samples that fluoresced before and after treatment and were not legible, as it allowed the text to be seen. This is a novel way of using luminescence that has not been documented previously.

When more hydrogen peroxide was applied to the ink on paper, it was expected that degradation of samples would increase and therefore the amount of products would increase. In some samples this was true, however, as the results for the benzophenone products showed, they mostly peaked after nine applications of peroxide and their values decreased after this. An increase in H_2O_2 will lead to faster degradation of samples but only to a certain level. It has been recorded previously that there is a critical level at which H_2O_2 will be effective and if the concentration is too high, degradation actually becomes slower. This is due to "self-quenching" of the H_2O_2 as it competes for the hydroxyl radicals.[153,160]

Hydrogen peroxide has shown to increase the rate of degradation of the dyes and the degradation products; benzophenones and phenols have been detected, but it also appears to have a negative effect on the products. Hydrogen peroxide is a powerful oxidant and it was actually destroying any molecules that resulted from the degradation of the dyes as well as the dye compounds, which would explain why the products' values were low and sometimes were not present for very long, with large fluctuations in value over time.

The ballpoint ink samples showed signs of demethylation and deethylation, but not necessarily in the homologue order that would be expected. As was seen, benzophenone products were evident following the degradation of the dyes, as

were phenol products, which are due to the breakage of the central phenyl bond. They were not produced however sequentially as demethylation or deethylation but concurrently. In some cases these products were detected almost immediately following treatment. Hydrogen peroxide produces hydroxyl radicals and according to the literature, the primary attack by hydroxyl radicals is to the chromophore.[158] Research has shown that when TiO_2 was used to photocatalyse Basic Violet 3, there were two competitive processes, N-demethylation and destruction of the conjugated structure.[158] Results from this current study have shown that the processes were occurring simultaneously. Previous research has only been carried out on dye standards and specifically with regards to removing the dyes from wastewater for environmental reasons. No research has been published that has looked at dyes solely for ink purposes.

Results to this point have agreed in some parts with the literature that hydrogen peroxide will affect the degradation of inks and dyes and colourless degradation products have been detected on the paper which could be used to re-visualise faded entries.

Hydrogen peroxide is just one bleaching agent that can be used to fade ink, another is domestic bleach which contains sodium hypochlorite.

5.5.3 Bleaching with Sodium Hypochlorite Bleach

When the samples were treated with sodium hypochlorite bleach some visual fading occurred, and some degradation products were detected. With the addition of 5% household bleach that has a sodium hypochlorite content of less than 5%, all standard dyes had some degree of fading within a couple of days, but Basic Violet 1 and 3 had the greatest difference in their colour. The successive degradation and subsequent production of homologues seen with photofading was not as apparent when bleach was used. Homologues were produced but there did not appear to be any particular order for their production. Degradation was occurring as different products were detected, but the lower homologues such as m/z 288 never had large values. The lack of lower homologues shows that with 5% bleach, the degree of degradation was less compared to the addition of peroxide or light. Differences were detected between Basic Violet 1 and Basic

Violet 3 that were highlighted in the aging curves. It appeared that the rate of degradation was faster for Basic Violet 1 than for Basic Violet 3, which was consistent with the results from photofading and peroxide bleaching.

In addition to demethylation and deethylation, the central bond can be broken as part of the degradation process to form benzophenone products. These were detected following bleach treatment for Basic Violet 1 and 3. The relative proportions were much higher for Basic Violet 1 than for Basic Violet 3 indicating the amounts detected were higher, so more has been produced which supports the results that the degree of degradation was greater for Basic Violet 1 than Basic Violet 3. Over the 40 days m/z 254 was the predominant peak for Basic Violet 3, although all products began to increase steadily. M/z 254 continued to increase whereas the other products decreased after 28 days. For Basic Violet 1, m/z 254 was also the dominant product, but m/z 240 continued to increase throughout the time as it was being produced by degradation. This could show that Basic Violet 1 was ahead of Basic Violet 3 in terms of the degradation process if they were following the successive homologue process. Phenol compounds were detected for the Basic Violet dyes but in differing amounts. The values for Basic Violet 1 at 6% were higher than Basic Violet 3 at 2% which was consistent with an increased degree of degradation.
There is no information available which refers to the reactions of inks with bleach, but there is some information about the reactions of some dyes in wastewater with sodium hypochlorite, but the conditions and dyes used for that research were different to those used in this research. It was observed that bleached seawater using sodium hypochlorite can oxidise pollutant dyes and remove the colour from the water.[161]

When light was used in combination with bleach, the degree of degradation was increased for all dyes which was consistent with peroxide bleaching and light. There were increased values for dye homologues and evidence of successive production seen with photofading was observed. There were also increased values for benzophenones and phenol products for all dyes tested. What was different to previous results was the greater degree of degradation for Basic Violet 3 over Basic Violet 1. Previously Basic Violet 1 had always had the greater degradation, but under these conditions, Basic Violet 3 did.

On paper, visible results were seen from hypochlorite bleaching of the ink and the fading of the inks was not uniform, showing that the degree of degradation for different inks and therefore different dyes was not the same, which was consistent with peroxide bleaching. The results of bleaching on paper were similar to the dye standards, in that few homologues were produced. Degradation was occurring as values of base peaks were decreasing after treatment but values for homologues were not increasing, and the main dye component remained the most abundant component during the analyses. An interesting observation has been the apparent lack of complete degradation of the dyes when observing chemical data, even though visibly the colour has all but disappeared. When samples were no longer black following bleach treatment, the dyes were still detected in their original form, and there was a distinct lack of homologues for some inks. The degradation process that occurred due to bleach was different to the process that occurs with other chemicals. It is possible that the bleach was reacting with other components of the ink, and not just the dyes. This may explain why the dyes were behaving differently to comparative inks under other conditions. It is also possible that the degradation of the dyes was only partial, and not complete as found by research on wastewater.[162]

The ballpoint ink samples showed signs of demethylation and deethylation, but not necessarily in the homologue order as expected. Benzophenone products were present following the degradation of the dyes which were produced due to the breakage of the central phenyl bond. They were not produced after demethylation or deethylation but at the same time. In some cases they were detected almost immediately following treatment, indicating that the degradation processes were occurring simultaneously.

No phenol products were detected for any of the ink samples on paper following bleach treatment, which was inconsistent with the previous results. However previous research looking at colour removal with sodium hypochlorite of dye wastewater also found that phenols were not produced in the reactions.[162] No research has been found that has documented the effects of sodium hypochlorite bleach on dyes specifically with a focus on ink.

Results have shown that bleaching (whether from sodium hypochlorite or hydrogen peroxide), or photofading will affect the degradation of inks and dyes and in different ways. Degradation products such as benzophenones and

phenols have been detected for the different conditions which may be beneficial for discrimination purposes.

Chapter 6 Discussion and Conclusions

This thesis has examined the aging processes of inks as they fade using Atmospheric Pressure Chemical Ionisation Mass Spectrometry for discrimination purposes. This is a different and novel focus from other research in the area, which concentrates on establishing the age of inks for investigative purposes. Whilst the dating of inks is of huge importance to the field of questioned document examination, there are many factors which influence the reliability of dating documents (such as storage conditions and temperature), therefore additional investigative solutions would be useful.

Document fraud covers a variety of situations, including the deliberate alteration or erasure of part or of a whole document. This project studied the theoretical and practical aspects of the chemical processes that dyes in ink undergo during aging and fading, under a range of different conditions. Natural and artificial aging using light, hydrogen peroxide and sodium hypochlorite bleach were used to deliberately fade ink entries to replicate cases of fraud. Aging of inks has been studied however, and APCI-MS was used in this project to identify the components in fresh ballpoint ink samples and dye standards in order to identify the degradation products produced during the stages of fading. For the examination of inks, APCI-MS has proven to be a sensitive, reproducible, rapid and easy to use analytical technique that requires minimal sample preparation and provides useful information about the compounds found in inks. However, the APCI mass spectra were complex, and full interpretation of the results was made difficult by the many unidentified peaks, but this is also the case for other well-known techniques such as Electrospray Ionisation Mass Spectroscopy.

Samples have included standard dyes, dye mixtures in solution and inks on paper subjected to photofading, hydrogen peroxide bleaching and sodium hypochlorite bleaching. The degradation to all these samples has been monitored and the identification of degradation products has been achieved using molecular mass values obtained from APCI-MS.

All of the conditions used to fade the inks had a different effect on the visual appearance of the inks and the degree of fading was different for all samples in all conditions.

Table 7.1 shows the most and least faded of all of the ink samples on paper for all of the conditions.

Table 6.1 Table to show the most and least faded ink samples for all conditions tested

	Most Faded			Least Faded		
	Photofading	**Hypochlorite**	**Peroxide**	**Photofading**	**Hypochlorite**	**Peroxide**
Blue	Woolworths	Woolworths	Woolworths	Bic	Staedtler	Staedtler
Black	Bic	Papermate	Staedtler	Micron	Micron	Bic
Red	Bic	Micron	Staedtler	Micron	Staedtler	Micron

It was clear to see that the Woolworths blue ink was the least lightfast ink of all the blue samples as it was the most faded in all conditions. For the other colours, for each external influence used, a different ink was the least lightfast, which highlights that the chemicals and light were affecting the inks in completely different ways.

The results from photofading clearly showed that light enhanced the degradation of dyes and inks whether in solution or on paper. This is because the dye molecules absorb energy from the light (photons) and if the amount of energy equals or is more than the activation energy for the molecule it will become an electronically excited state which renders it available for reactions. These reactions might be with oxygen which would lead to photo-oxidation. These reactions are consequently changing the structure of the dye molecules such as the loss of $-CH_3$ groups which will change the colour observed. This is due to the new molecules absorbing different wavelengths of light to the original molecules and therefore what is reflected is a different colour and can be observed as fading. Demethylation results in hypsochromic shifts in the absorption bands of the dye molecules.[164] The new molecules formed may have enough energy from photo-oxidation to react repeatedly, which causes quick reactions and enhanced rates of degradation.

The results from photofading revealed clear successions in the production of the dye homologues for all standards and ink samples, supporting the theory of

demethylation as a degradation pathway. However, benzophenone products were also detected before demethylation was complete indicating that degradation was occurring via two simultaneous pathways and not successive pathways. This actually makes sense, because if demethylation was complete, no -CH_3 groups would be present on any homologues, but there were between one and four -CH_3 groups present on the benzophenones detected.

Results showed that Basic Violet 1 had a greater degree of degradation than Basic Violet 3 which was consistent across all conditions, apart from sodium hypochlorite bleach with light. The degree of degradation was different for all dyes which is due to their different structures and reactions they undergo. The degradation is also not continuous. At some point it reaches a limit and slows down significantly which can be explained by the lack of molecules to react due to their previous reactions, but also by a lack of light. As the λ_{max} of the molecules shifts to shorter wavelengths such as 500 nm, the intensity of the light decreases, so there is less light to absorb, if the molecules even absorb at this range. Also less singlet oxygen will be produced by the dyes, which reduces the rate of degradation in other dyes. If the molecules do not absorb light, such as the benzophenones, or are in a region of the spectrum where there is no light to absorb, they will be stable and may not demethylate into their homologues. Degradation however does not stop if the light is removed, as seen by Grim, oxidative demethylation continues until the coloured dye components e.g. m/z 372 to m/z 288 for Basic Violet 3, are completely reduced.[26] Results also showed that the dyes may behave differently when there is more than one dye present in a mixture (just like in an ink). This is due to the synergistic effect that dyes have on each other and how they compete for the same energy.

The samples exposed to natural light were not kept in a darkened room, or an uninhabited room. Light illuminating the ink entries from behind could be possible and may have had an effect on the entries. A normal sheet of white office paper is translucent, so light from the bulbs in the room may have had an additional effect on the aging of the ink samples.

The results from this study were obtained in one instance by light exposure, and specifically visible light. Natural sunlight was used to fade ink samples on paper,

and artificial daylight was used for dyes in solution. The wavelength of the light used to fade ink will affect the degradation process. Ultra violet light is known to induce fading more than visible light because UV light has higher photon energy than visible. This would lead to more reactive electronic excitations of the dye molecules and faster aging. Forensic investigators should be aware of the different influences that light can have and this would need to be taken into account if someone had deliberately faded a document with light.

When hydrogen peroxide was used to deliberately bleach the samples degradation was enhanced compared to no bleaching, but it appeared that the degradation was not as quick as photofading. When light was used in combination with hydrogen peroxide, the rate again appeared to be faster for all samples. Hydrogen peroxide is a strong oxidising agent and can be easily obtained. The hydroxyl radicals can react with dye molecules by electrophilic addition to produce intermediates which can cause discolouration and degradation of samples. Discolouration does not mean complete oxidation though as it has been seen that even with substantial loss of colour, dye molecules were still detected. The successive production of homologues observed with photofading was not observed under these conditions. Degradation of the main dye component was seen, but the homologues were not produced in the same quantities as with photofading. It was seen how benzophenone products were detected quite early in the treatment which is attributed to the knowledge that primary attack by hydroxyl radicals is to the chromophore structure with leads to breaking of the central bond.[158] This may explain why the homologues were not detected as the breaking of the central bond occurred before they were produced, and after the breakage they were unable to be produced. Benzophenones and phenols were produced early, but they did not remain present or in high quantities for long which is believed to be due to their destruction by the hydroxyl radicals.

Sodium hypochlorite bleach was also used to deliberately fade the samples. Results showed that bleach did enhance the degree degradation for the dyes and with added artificial daylight exposure, it increased more. Like with hydrogen peroxide, no obvious succession in dye homologues was seen and the actual values for the homologues remained low throughout the analyses even though

visible fading had occurred. The main dye components e.g m/z 372 for Basic Violet 3, remained the most abundant components in solution and on paper even after treatment with bleach. Benzophenone products were observed following treatment, not after the complete destruction of the dye, but at the same time. This was consistent with the other conditions whereby the degradation pathways were occurring simultaneously. It would appear that for hydrogen peroxide and sodium hypochlorite bleaching, the more dominant pathway is via breakage of the central bond, as opposed to de-methylation as benzophenones were detected more than the dye homologues. The reactions occurring to cause the fading are based on the same theory as hydrogen peroxide and photofading. The bleach oxidises the chromophore bonds which changes the structure so that it no longer absorbs the same wavelengths of light and therefore the colour changes. Benzophenone products were detected as with the other treatments, and as seen previously they appeared early in the treatment but did not really increase in abundance over time which suggests they were being destroyed probably by the bleach. Phenol products were detected for some samples but not at all for the ink on paper samples. Previous research on dyes that were treated by sodium hypochlorite found that the mechanism for colour loss was by destruction of the chromophore but it was only partial,[162] this would explain why phenols were not detected as they had probably not been produced if degradation had not proceeded to this stage. No previous research has been carried out deliberately fading ink with hydrogen peroxide and sodium hypochlorite bleach and the results showed that inks were affected in different ways by different chemicals and light and these results, specifically the ink on paper, are new to the area of forensic document analysis. This study has provided new information about the differences that chemicals used in chemical erasure of inks and photofading can have on a document. This could be of much value to document examiners with regards to dating as not all samples faded at the same rate under different conditions, so considerations need to be made when forming opinions. Also as the products were different specifically a lack of phenols from sodium hypochlorite bleach, an examiner would be able to determine the method of chemical erasure.

It was observed for some of the dye mixtures how the behaviour of the dyes can be influenced when in combination with other dyes. Basic Blue 26 was used in combination with Basic Violet 1 and 3 which replicates typical blue ballpoint inks. The wavelengths at which these dyes absorb light are quite close, λ_{max} for Basic Blue 26 is 610 nm and for Basic Violet 3 is 590 nm. This means that they will compete for the absorption of the light and quench the degradation of the other dyes in the process. The degradation of Basic Blue 26 appeared quite stable when alone but when in combination with Basic Violet dyes their degradation was much greater. Another reason for increased degradation in mixtures is that singlet oxygen can be produced by dyes particularly in light which can then attack other dyes.[66]

Optical examination of the inks using a Video Spectral Comparator 5000 allowed a degree of discrimination between the samples based on infrared absorption and reflectance and infrared luminescence, but no differences were observed in the infrared absorption or reflection results between fresh and old inks under any conditions. There were differences seen however in the IR luminescence findings. Prior to any treatment eight of the ink on paper samples exhibited fluorescence and after all treatments they still fluoresced, but after photofading five further samples fluoresced. Hydrogen peroxide bleaching induced fluorescence in one extra sample, and sodium hypochlorite bleaching induced fluorescence in four other samples. These results clearly show that the different conditions are affecting the inks in different ways as they do not respond the same when treated with different chemicals. The ability of a sample to emit fluorescence is due to the molecules within that sample absorbing radiation and being promoted to an excited state and returning to the ground state by remitting the energy at a longer wavelength. The degradation processes used in this research clearly affect the structure of the molecules present in the inks which affect the absorption characteristics of the molecules which leads to the changes observed in the fluorescence. Inks can contain different components that luminesce and it may not necessarily be the dyes. One paper has been found whereby fluorescence was induced in thirty ink samples by steam,[165] but they could not explain the reasons for this. They also tested samples with light and sodium hypochlorite bleach and some samples still fluoresced but no new samples did unlike this current research. They believed that the reason for fluorescence after

treatment was because the component in the ink which gives the fluorescent effect penetrated to a greater depth in the paper than the colouring component. No other literature could be found to explain the importance of this. Much research has been carried out particularly for environmental purposes which look at hydrogen peroxide and chlorine reactions with dyes, but nothing has been published that specifically looks at the dyes from a forensic ink perspective. The ability to alter a compound's optical characteristics is of huge importance to the forensic community. Discriminations between samples take place based on these characteristics, and an incorrect decision could be made which concludes inks are different when in actual fact they have been altered over time naturally or deliberately. Far more research is needed in this area to fully understand all the different factors that could affect luminescence properties and lead to incorrect decisions by examiners.

Re-visualisation of some faded samples was successful using IR luminescence. This has not been attempted previously. The results were different for each treatment that the samples were subjected to and in the cases of hydrogen peroxide and sodium hypochlorite bleach, this may actually be chemiluminescence rather than luminescence as the excited state would be due to the reaction between the chemical and the sample, rather than absorption. Further research in this area is needed to clarify the mechanism for the luminescence, if it is due to changes occurring to the structure of the ink due to the chemical which causes changes in the absorption, or if the chemical reaction is itself the cause of chemiluminescence. Chemiluminescence is currently not known to be used in document examination.[44] This may be of huge importance to document examiners as it may provide a means of viewing faded entries or erased entries, but the idea of adding chemicals to documents is not one that is favoured by scientists as it would not be classed as non-destructive. A lot more research is required in this specific area before it could be a legitimate method of examination, but research from Raman spectroscopy and more specifically surface enhanced Raman spectroscopy (SERS) may help to support the use of chemicals on documents. In SERS a colloid is applied to the surface of the sample to enhance the Raman signal and the technique has been classed as non-destructive.[130] It is also now a widely accepted technique in document examination.

At time zero, i.e. before aging has begun, the relative proportion (RP) value was seen to be below 100% for some dyes. RP 372 values from the 19 ballpoint pens that were determined prior to aging ranged from 26% to 51% for the blue pens and between 27% and 80% for the black pens and thus the aging behaviours will differ accordingly. An ink that contains a dye with only 27% relative proportion could reach 0% more quickly than an ink that was 100% at the start, but it would depend on the other components present in the ink as they can influence the rate of degradation as seen with the mixtures. It is therefore extremely important that when mass spectra of inks are being interpreted for dating purposes, all possibilities need to be considered. Just because there is less dye present when a sample is analysed does not mean that it has degraded more or is older than another sample that contains more dye. Ideally the initial composition of an ink would be known for a correct interpretation of the mass spectra to be made, but this is usually not possible, therefore it must be made clear to document examiners that when ink dating conclusions are to be given many factors need to be taken into account.

APCI-MS mass spectra were used to characterise pure dye standards and analyse ink samples by the presence of molecular ions which was consistent with previous authors using electrospray ionisation mass spectrometry.[32,136,166] The results from this current research has shown that the novel approach of using APCI-MS for this type of sample, namely inks, can be successful in discriminating between samples and identifying specific dyes present in the samples and it provides an alternative analytical technique available to examiners.

In the spectra produced from APCI-MS there were at times additional unknown signals present which may have overlapped with known signals. This is because inks can contain additional dyes and pigments which remain unidentified. Even with the availability of a mass spectrometry library, it is impossible to identify all components due to the large number of possibilities. The signals from these components can interfere with the signals from the known components. In this research only the products clearly related to the dye in question were taken into account when calculating RP values. This means that the results from this research may not be as accurate as would be liked, but the results were treated consistently across all samples analysed.

All APCI-MS analyses in this study were carried out in the positive mode. Therefore only positive ions were detected and identified. No negative mode analyses were undertaken. Some inks will contain compounds that produce negative ions such as the anionic dye Metanil yellow. It is likely that some of the pens will have contained different compounds that were not detected due to this reason. Further research needs to be carried out to determine the negative ions in these samples as they may provide other alternatives for re-visualisation of the faded ink entries.

Before finalising the methodology used in this research, different variations were tested. Initially the extraction solvent chosen for APCI-MS analysis was benzyl alcohol as suggested by Ng *et al.*[32] The solvent did extract the ink off the paper into solution, however the signal from the solvent was very high and was masking the signal from the actual ink samples, therefore methanol was chosen as the extraction solvent which was equally as good at extracting the ink off the paper into solution. Sonication was used following extraction even though there are differing reports on its success in enhancing the extraction process,[104,44] but it was felt that it did not hinder the process so would be used. Originally all ink extraction samples were prepared at the same time and then were analysed one at a time, but due to the time taken to analyse one sample multiple times and to run multiple blanks between each sample to ensure the equipment was free of contaminants, some of the samples would evaporate before they had been analysed therefore the samples were extracted as were required.

As has been mentioned briefly there were instances where contamination of the MS spectra was a problem. This is due to contamination of the equipment. The system is used heavily by numerous researchers, and to analyse a multitude of samples. Unfortunately not all samples were cleaned from the system after usage. Also it appears to be an inherent issue with this type of equipment that contamination will occur even if procedures are in place to prevent it and *Thermo* provide lists of common contaminants.[167] Contaminants can arise from several sources; solvents, columns, plastic containers, pipette tips, filter membranes, but the most popular contaminants are plasticisers such as dioctyl phthalate with m/z values of 391 and 413 which appeared frequently in the mass spectra of this study and regularly were the most abundant components detected. Background

data was subtracted from the total ion chromatogram of each sample, but was not completely effective in removing the contaminants from the final spectra.

When peroxide and bleach were applied to the paper surface, originally this was done using a medical swab. It was noted that the paper surface began to be affected by the rubbing of the swab and was causing abrasions and therefore damaging the surface. Following on from this, a child's paint brush was used. No damage to the surface was seen from application with the brush. The stroke movement of a brush appears to be softer on the surface than the wiping motion from using a swab. With either technique, the amount of chemical applied was not measured only the number of applications. The writing was covered only once for each application, and was allowed to dry before each further application. With each application, a relatively small amount of chemical was applied because if too much was used, the paper became very wet. Visible changes that occurred included a decrease in the smoothness of the paper surface where the chemicals had been applied. The paper became slightly crumpled in those areas and the paper edges curled. Also there was a change in the colour of the paper where the chemicals were applied, it was no longer white but yellow.

Not all variables were controlled for this research such as temperature, light and humidity, but all samples were subjected to the same conditions so the results are viable. Temperature and light were recorded for periods of the study, but humidity was not. It is known that light and temperature will speed up the degradation process, but little research has been carried out as to the effect of humidity. The pH of the paper used may be affected by high humidity conditions as paper has a basic reaction with water and consequently may influence the degradation.

The influence of paper was not a focus of this research. All samples were applied to the same paper throughout the project, so all results will be consistent. How paper was affected by the conditions; light, sodium hypochlorite bleach and hydrogen peroxide was also not analysed. The paper will have undergone some amount of paper decay during this time which may have a bearing on the degradation of the inks, but as this variable was kept constant, the results can be

considered valid. Further research is needed to discover the true effects on the paper and consequently on the inks.

Also with regards to paper, some substances such as TiO_2, which are used in the manufacturing process of paper, can catalyse the degradation of dyes.[61] Titanium dioxide is also the most common compound used to photocatalyse organic chemical reactions.[149] This may have occurred in this study, but the paper was not analysed to confirm if any of these compounds were present, and as the samples were all on the same paper, if catalysis occurred, it should have affected all samples, but this would need to be determined.

It was noted that as the ink faded on paper, it did not necessarily fade consistently. In certain parts of the ink line, ink remained as a sort of ink blot. There are two reasons for this. One is due to the ink thickness and from when and how the ink was deposited on to the paper surface. It can happen that when a ballpoint pen moves across the paper, at times thicker deposits of ink are left, it is an unfortunate feature of the ballpoint system. The second reason is due to the nature of the paper surface. Paper does not have a smooth flat surface due to the manufacturing process and the way the fibres are bound together. This means that the ink will not be deposited on the surface homogeneously. When samples were taken for analysis, they were not taken from the areas of thicker ink, but it is important to be aware that if samples had been taken from the thicker ink sites, the degradation products may have been different, as they depend on the dye concentration and a thicker ink means a higher concentration. This is extremely important in terms of forensic analysis and ink dating.

Referring to the objectives of this project as highlighted in section 1.5,

- The examination of a range of ballpoint pen inks of different colours, representative of those available in the UK using Atmospheric Pressure Chemical Ionisation Mass Spectrometry has been carried out
- The investigation of the chemical processes that occur and the ultimate fading products that are formed after the deposition of ink onto a solid substrate and when subjected to light, hydrogen peroxide and sodium hypochlorite bleach has been carried out

- The investigation into the aging processes of dye standards in solution when subjected to light, hydrogen peroxide and sodium hypochlorite bleach has been carried out
- The investigation into the aging process of dye mixtures in solution when subjected to light, hydrogen peroxide and sodoim hypochlorite bleach has been carried out

Further work is required to corroborate the results of hydrogen peroxide bleaching, sodium hypochlorite bleaching and photofading, especially with regards to fluorescence. Not all of the samples fluoresced, and the component which caused the fluorescence has not been identified as there were no common dyes in every ink. More samples need to be analysed to determine what the possible fluorophores could be.

In this project the actual rate of degradation was not calculated, only the degree of degradation based on ion count data. For more accurate results, calculating the rate of degradation under all conditions would be extremely beneficial to a document examiner and may provide more robust data for casework where aging is a factor. Further research is needed to determine which order of reaction would be most suited to these samples and conditions, but as the rate is influenced by the concentration of the dye, zero order kinetics would be inappropriate.

Only one sample of each colour from each manufacturer was tested in this study, and only a maximum of seven pens per colour (usually four). More samples of each colour are required and more colours from the same manufacturers are needed to confirm the results and determine if there is consistency for larger numbers.

This study only examined ballpoint pen inks as they are the most popular pen used on a daily basis. However, gel pens are becoming more popular. Similar tests could be carried out on different pen types such as gel and rollerball to see if they are affected by the chemicals and light in the same way, or if they produce different, but useful results.

Paper was a controlled factor in this research, but the effects of the paper on the inks, and the effects of the chemicals on the paper could not be controlled. Results for this study should be consistent, but investigations need to be made into how the bleaching process may have affected the paper and if this contributed to the fading of the inks.

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