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Black Writing Ink Analysis By Direct Infusion Electrospray Mass Spectroscopy

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BLACK WRITING INK ANALYSIS
BY DIRECT INFUSION ELECTROSPRAY MASS SPECTROSCOPY

by

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ABSTRACT

An optimized method of extraction, an instrumental analysis method and data analysis was proposed for black writing inks based on direct infusion electrospray-mass spectrometry (ESI-MS). The sampling and analysis method is both minimally destructive and able to assess differences in inks from a reference collection of thirty ballpoint, gel, and rollerball inks. The methanol extracts of ink on paper samples were analyzed with three direct infusion (ESI-MS) methods. Each method varied scan voltage negative and positive, ESI fragmentor applied voltage (+120V, +0V, and -120V), and mobile phase additive. Direct infusion ESI-MS analysis, followed by pair-wise comparisons of the observed ion data in binary form allowed inks to be distinguished from each other. The photobleaching of the dye Basic Violet 3 (BV3) in ink-on-paper samples was examined to determine the use of degradation products as a marker of the age of the writing sample. The extent of photobleaching of BV3 was determined using several illumination sources. Pair-wise comparison of observed ion data was able to distinguish 29 of 30 ink samples using the combined three instrumental methods. Out of 435 pair-wise comparisons 429 pairs could be discriminated from each other using the combined three methods. This is a 98.6% discrimination with the combined analysis scheme.

To my mother and father:

“Man’s mind, once stretched by a new idea, never regains its original dimensions.”

— Oliver Wendell Holmes

ACKNOWLEDGMENTS

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CHAPTER ONE: INTRODUCTION

While personal and business correspondence has changed dramatically in recent years due to the usage of computers, electronic mail (email), fax machines, e-forms, text messaging, and digital signatures, the use of a physical written signature as a legal binder is still commonplace. Despite the advances in correspondence, writing with some form of writing instrument such as pen, pencil, or marker is still popular. A physical signature written on a physical document is used to prove or affirm the identity of the signer on such legally important documents as checks, contracts, bills of sale, insurance policies, wills, and birth/death certificates. At times, the authenticity of such legal documents comes into question and may require additional analysis to assess claims associated with such important documents.

The current economic climate has increased the threat, if not the occurrence, of fraud. Fraud is defined as a crime that involves falsified documentation presented with the intent to deceive another for profit. White-collar criminals commit crimes of fraud that cost Americans more dollars than material theft each year. News headlines are full of details regarding fraud-type white-collar crimes from the Enron scandal to Barnard Madoff's Ponzi scheme. Other notable fraud cases involving questioned document evidence include Michael Jackson's Will and Howard Hughes' so-called "Mormon Will." In these cases, questioned documents (QD) examination was required to uncover an attempted fraud. A complete QD examination should include several separate types of analysis; for example, personality traits assessed by handwriting analysis, signature comparison; trace evidence and ink analysis. The scope of this research is the detailed ink analysis portion of questioned document examination for the purpose of differentiating ink extracts using direct infusion ESI-MS analytical methods.

Richard Brunelle of the U.S. Bureau of Alcohol, Tobacco, and Firearms (ATF) identifies the reasons for an ink analysis in a total document examination methodology[1];

1. “To compare two or more ink entries to determine similarities or differences of inks which can provide information as to whether certain entries could have been added or altered.”
2. “To determine whether two or more ink entries consist of the same formula ink which provides a lead concerning whether the entries could have been written with the same pen.”
3. “To date the ink entries to determine whether documents have been backdated.”

Instrumental methods are objective and independent of analyst experience level, which stands in sharp contrast to many of the non-instrumental document analysis methods. The National Academy of Sciences (NAS) report on the forensic sciences suggested that the Questioned Document Examination Section place less emphasis on methods that rely primarily on the assumption that an individual’s handwriting is measurably unique [2]. The NAS concluded that uniform scientific terminology should be adopted for describing, interpreting and reporting of QD analysis results. The NAS further states that ink analysis was a proven method of examination, based in analytical chemistry, and has a system in place to describe theory and practice of operation.

In the DAUBERT v. MERRELL DOW PHARMACEUTICALS, INC., 509 U.S. 579, The United States Supreme Court said “When faced with a proffer of expert scientific testimony under Rule 702, the trial judge, pursuant to Rule 104(a), must make a preliminary assessment of whether the testimony's underlying reasoning or methodology is scientifically valid and properly

can be applied to the facts at issue. Many considerations will bear on the inquiry, including whether the theory or technique in question can be (and has been) tested, whether it has been subjected to peer review and publication, its known or potential error rate and the existence and maintenance of standards controlling its operation, and whether it has attracted widespread acceptance within a relevant scientific community."[4]

Until quite recently, forensic document examiners preferred to only use non-destructive (ND) ink analysis methods since document destruction was not considered a workable option. Non-destructive methods often rely on analysis of optical properties which require the use of alternate light sources such as ultraviolet, infrared, or oblique lighting. These tests attempt to exploit optical properties of the ink, paper, and their interactions to visually discriminate ink formulations without destroying the document. Many of the properties that are used with ND methods allow an examiner to reach a conclusion that is concentration dependent. Optical ink analysis methods are subject to interference from interactions of ink, paper, and other chemicals. The interpretation of "data" from ND methods is subjective in nature and dependent on the examiner's experience. The only way a document examiner can acquire experience is on the job training and casework; there is no educational program specific to the scientific examination of questioned documents. [5]

Ink

Commonplace colored items include, fabrics, textiles, building materials furniture, cosmetics, electronics, and writing media and all available in a spectrum of colors. The colors that we perceive are imparted to these items by synthetic or natural compounds called colorants. Many compounds that are used as colors in textiles can also be used as ink colorants.

Ink used in writing pens is comprised of two basic components: colorant and vehicle. In general, inks are either colored with organic dyes or insoluble inorganic pigments. The colorant is then dispersed into what is collectively called the vehicle. As the name implies, the colorant imparts the color to the ink mixture. In order to achieve a particular color, a manufacturer may mix two or more pure colorants to create the desired color. The vehicle portion of ink is comprised of lubricants, flow control agents, polymers, and other ingredients added to manipulate the ink properties according to the manufacturer's needs. The solvent, which is in the vehicle (or is the vehicle), allows the ink mixture to be deposited and flow on the paper surface in a relatively predictable manner. Some common solvents found in pen inks include but are not limited to: ethylene glycol, 1,2-propylene glycol, 1,3-butylene glycol, glycerin, phenoxyethylene glycols, benzyl alcohol, ethylene glycol monomethylether, and diethylene glycol monomethylether[1]. When one writes with a writing instrument, ink from the pen (inks) reservoir is deposited on the writing surface so the writing can be read at a later time. The notable exception is "invisible" ink, which requires a developer such as citric acid and will not be considered further in this work. Ink is deposited as a thin film on the surface of the paper. Volatile components diffuse and adsorb at a faster rate than the colored portion, as they are not intended for permanent inclusion in ink deposits. The nature of the imperfections of mass manufacturing of pens insures that an ink deposit is never present in a consistent or predictable manner. As ink begins to dry, the volatile portions begin to vaporize and to disperse into the air and diffuse into the paper surface. The presence of volatile components in black ballpoint (BP) inks was examined to assess persistence. It was concluded from mass spectral data that after one week, no detectable volatiles remained in the samples[2]. Ink may also contain resins and

species, which can polymerize with exposure to the air. Three types of ink pens commonly encountered are ballpoint (BP), gel pens(GP), and rollerball (RB).

Table 1: List of pens used and their manufactures

MSDS listing "Product Name:"	Manufacturer	Other pens using a similar formulation	Listed ingredients
Paper Mate Erasable	Sanford Corp	Replay, Replay Futura, EraserMate, Eraser.Max	Solvent:naptha [64742-89-8], xylene [1330-20-7], ethylbenzene [100-41-1]
Paper Mate Ball Point Pens and Refills	Sanford NA	Saga Hex, Saga Stick pen, Saga Retractable, Comfortmate Stick Pen, Comfortmate Retractable, Comfortmate Grip Retractable, Dynagrip Plus, Dynagrip RT50, Flexgrip Ultra Stick Pen, Flexgrip Ultra Retractable, Mystix Fashion Stick Pen, Pogo, PhD, Profile Regular, Profile Slim, Silhouette, Slinger, SureGrip Retractable, Visibility, WideMate, X-Tend Stick Pen, X-Tend Retractable	2-Phenoxyethanol [122-99-6], ethoxydiglycol [111-90-0], resins, dyes, additives
Uni-ball Vision Pen	Sanford NA	None listed in MSDS	Water, propylene glycol [57-55-6], pigments
Pentel BLD 66 Tetra	Pentel of America	None listed	None listed
Pentel K116 Hybrid Gel Grip Product # K116	Pentel of America	None listed	None listed
G-2 Refill Ink: BG25/7R	Pilot Corp.	(G25/7, G67, BDGL7, BDGG7, BEXG)	Ethylene Glycol [107-21-1], Triethanolamine [102-71-6]
Energel Refillable Gel Roller Pen	Pentel Co., LTD (Japan)	None listed	Ethylene Glycol [107-21-1], Diethylene Glycol [111-46-6], Glycerol [56-81-5]
Sharpie Fine Point Permanent Marker	Sanford Corp	None listed	Dyes, n-propanol [71-23-8], n-butanol [71-36-3], diacetone alcohol [123-42-2]
GI100-Black 07	YOU&I Corp.	None listed	C.I. Pigment Black 7 [1333-86-4], Thickener [11138-66-2], Polyethylene Glycol [25322-68-3], Glycerin [56-81-5], Surfactant [39464-66-9], Triethanolamine [102-71-6]

Figure 1: Black ballpoint ink on plain white copy paper, enlarged to show detail, 30-minute drying time

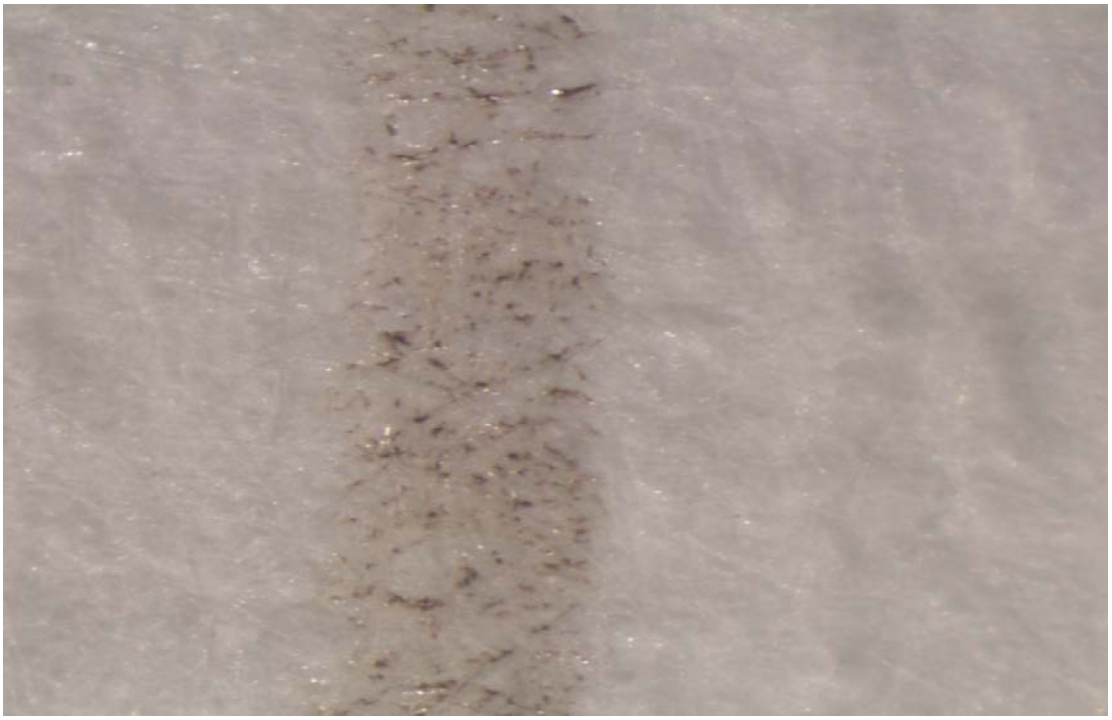

Figure 2: The same ink line after three days of continual light exposure, with a common desk lamp

Ballpoint pen inks are the most widely distributed of the commercially available pen inks. They contain synthetic dyes and a glycol based solvent. Before 1950, ballpoint pens were produced with an oil-based vehicle, which was later switched to a glycol-based formula that was reputed to be safer and able to be applied more evenly on the paper. Ballpoint pen inks, which are cheaply mass-produced, are subject to photobleaching over time. Figures 1 and 2 compare the same ballpoint ink line before and after 72-hour exposure to a common desk lamp's output. Gel pens were developed with insoluble pigments to provide a color that does not fade under normal conditions[3], as seen with ballpoint inks as in Figure 2 above.

The gel pen, introduced in the U.S. in 1989, typically contains a finely ground insoluble pigment as the colorant with little or no volatiles, see Figure 3 for a typical gel ink (deposition) on paper. A blue gel pen was used in the photomicrograph to highlight the deposition characteristics of a typical gel ink. One black GP ink formulation, U.S. Patent, No. 5,993,098, contains, carbon black, acrylic resin molecular weight 5000 g/mol, aminomethylpropanol, alkylphosphates, xanthium gum, glycerin/glycerol, propylene glycol, and deionized water[4]. A blue rollerball ink pen (see Figure 4) can contain a combination of dyes, pigments and additives depending on the needs of the manufacturer and the consumer. At present there is no single analytical scheme that provides an extraction and analysis of differentiation of all of the common ink classes. A significant portion of the published research on pen ink analysis has been conducted on BP inks, as they are the oldest of the commercially available ink formulations. The positive identification of unknown samples may require new methods and even new instrumentation. The best possible estimation of the composition of a given ink is based on the type of ink and the knowledge of ink formulations.

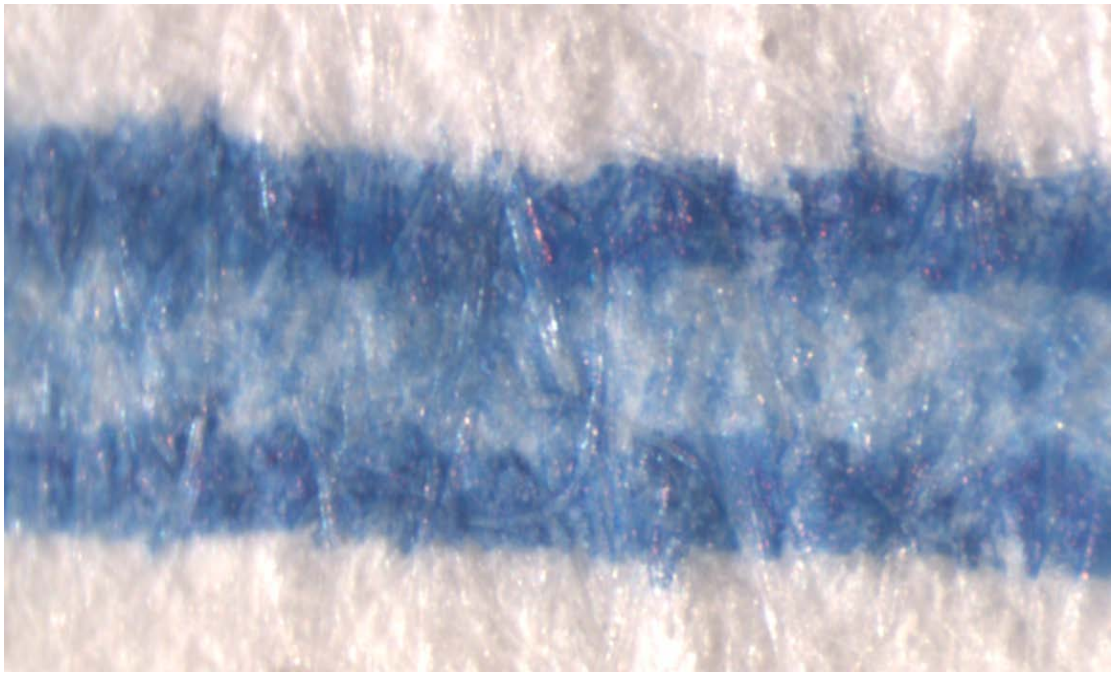

Figure 3: Blue gel ink on white paper

Figure 4: Black roller ball ink deposited on white paper

Since most ink formulations are held as trade secrets, the only published information concerning possible formulations are the Material Safety Data Sheets (MSDS) provided by the manufacturer. Compounds identified by MSDS sources found in some popular brands of ink pen formulations, the listed ingredients and other pens with “similar formulations” can be seen in Table 1. The listed ingredients identify some compounds used in several pen ink formulations. The compounds in the list were used to select standards for use with instrumental methods for comparison to ink sample data, see Table 2 for pens and inks used for this study.

Table 2: Pens used in study by number, name and type

Pen	Description	Type
1	Bic Cristal	Ballpoint
2	Mega SRX 500 Ballpoint	Ballpoint
3	Cross Ballpoint Pen Refills Med Blk	Ballpoint
4	Papermate Med pt Blk	Ballpoint
5	Pilot BP-S Med Blk	Ballpoint
6	Bic Ultra Round Stic Grip	Ballpoint
10	Tul gel retractable fine black	Gel
11	inc. Classic gels black	Gel
12	Parker Jotter (Gel Refill)	Gel
13	PentelEnerGel Liquid ink	Gel
14	Zebra Gr8 Gel	Gel
15	Uni-ball Signo (207)	Gel
16	Rose Art	Gel
17	Uni-ball Bold Jet Stream Sport	Rollerball
18	Uni-ball Deluxe Fine	Rollerball
19	Pentel Tetra Liquid Ink Fine	Rollerball
20	Pentel Hybrid Gel Roller K176	Rollerball
21	Bic Z4 Roller	Rollerball
22	Uni-ball Vision Micro	Rollerball
23	Bic Pro+ Ball Pen	Ballpoint
24	Bic 4 Color (Using only black)	Ballpoint
25	Pilot BP-X	Ballpoint
26	Zebra Ola Med	Ballpoint
27	Zebra Z-365	Ballpoint
28	Pilot Easytouch Retractable	Ballpoint
29	Zebra Z-grip Med	Ballpoint
30	Zebra F-301 compact fine	Ballpoint
31	Papermate X-tend BP	Ballpoint

Forensic Document Examination

Forensic examination of any questioned document will typically include microscopic and macroscopic examinations before proceeding to any test that consumes or destroys a portion of the sample. Microscopic examinations can be conducted with a stereomicroscope and a comparison microscope. The information provided by microscopic examination of a questioned document can help quickly identify the paper and class of pen used. Forensic document examiners perform other types of document analysis, such as handwriting analysis and deciphering hidden (decipherment) or visualization of obliterated writing[5]; however, those types of analysis are outside the scope of this research.

Ink Analysis

The goal of the QD section is to provide scientifically validated methods of document analysis. Many QD sections are accepting the new standards and adopting principles that will help to establish the discipline as more scientifically and legally defensible. Ink analysis is derived from analytical chemistry, which has accepted and validated methodologies. Analytic chemistry also provides strategies to implement new and novel analysis schemes. The National Academy of Sciences defines the objectives of ink analysis as: “An ink examination can have one of two objectives; class identification, for which the intention is to identify the ink formula or type based on a reference library of samples of inks, or by comparison of two ink samples to determine if they share a common origin[8].”

The ultimate goal is to allow potentially fraudulent documents to be investigated with scientifically based and validated methodologies. An effort to keep documents intact to preserve their evidential value is considered key; therefore methods of document analysis have

traditionally relied on non-destructive methods. Non-destructive methods of ink analysis do not assess ink formulation or an ink's chemical composition. In order for a more complete and scientifically valid method of document examination, some destruction of the document is required. As long as the amount of sample consumed is relatively small, the integrity and evidentiary value of the sample is preserved. The additional information provided by destructive means can help to answer questions posed by Brunelle (page 2).

There are several types of criminal activities that utilize an authentic document. Detection of these types of crimes may require ink analysis. An insertion is committed by inserting pages or passages that were never intended for inclusion in the document by the writer. A deletion involves something erased (or removed) from a document by chemical or physical means[6]. Addition forgeries are made up of items never intended for inclusion in the original document for example increasing the value of a check by adding extra numerals or manipulating the original writing in some manner without erasure. Detection of these alterations requires the use of a total analysis scheme, which begins with the least invasive and ending with those that consume some of the sample.

Non-Destructive Analysis Methods

The methods used in a forensic document examination can include destructive as well as non-destructive (ND) methods of analysis. Obviously, ND methods are preferred as they will leave the document intact and preserve the value of the document as a piece of evidence. However, a drawback to ND methods can be the limited amount of information and discrimination provided by some of these methods [7]. One ND method described in ASTM E-1422, the dichroic filter examination, is mentioned as a comparative technique.

Dichroic Filter Analysis

Dichroic filter examination utilizes two dichroic filters, which block the transmission of one portion of the electromagnetic spectrum, allowing another portion to pass through the filter. The sample in this type of examination is illuminated with a light that has the red and infrared portion removed with a band pass filter. The observer views the sample through a green filter [7]. The combination of these filters can accentuate the interactions of the sample with the blue and green portions of the electromagnetic spectrum. This interaction can create a situation where the optical properties of a sample will exhibit characteristics that may allow the analyst to conclude that samples do not have a common origin. Not every sample has unique qualities observable by dichroic filter examination, thus limiting this method to a preliminary examination before a more sensitive destructive method is applied. If the analyst could determine the answer to the question without having to destroy a document, they should work from less to more destructive methods. Paper typically contains UV brighteners which, when disturbed by erasure, may be evident with some non-destructive means[6]. A major drawback of the use of dichroic filters is that the analyst is limited to only a tentative identification of a questioned sample. Individualization and positive identification of inks is impossible with dichroic filter examinations. Some newer ND methods utilize an image digitalization device, and image processing software.

Digital Processing Methods

Hammond examined black BP pens by a digital image processing method [8]. The method uses a flatbed image scanner, followed by analysis with the LAB color mode of commercially available imaging software. A validation study of 44 pen samples created 990 pen-pair samples of which 28.5% were undifferentiated by the method [8].

When a simple or non-destructive test is not discriminatory or is inconclusive, more destructive methods such as gas chromatography/mass spectrometer (GC-MS) analysis may be required to provide more information.

Gas Chromatography/Mass Spectrometry (GC-MS) Analysis of Inks

Many of the non-destructive methods of ink analysis provide results that are subjective in nature. Other non-destructive methods are based on recognizing an easily identifiable class feature. For example, determination if a questioned ink sample is an oil-based ink, due to its solubility in organic solvents[11]. Many types of trace evidence analysis rely extensively on GC-MS methods. GC-MS analysis is useful with molecules, which are volatile in nature. An important limitation of this method is that some molecules can degrade at inlet and oven temperatures commonly used for GC-MS analysis (250-280°C). Analysis of molecules, present in a sample as salts, is not readily accomplished with GC-MS due to their insolubility in with organic solvents and the high vaporization temperatures of salts. GC-MS methods are reproducible, allow for good separation of volatile analyses, and some manipulation of analytical parameters by variation of column bonded phase composition. Typically, GC-MS analysis passes a stream of GC eluate into electron ionization (EI) source operated at 70 eV. This process ionizes neutral analyte molecules and creates characteristic molecular fragmentation patterns[12].

Electron ionization fragmentation patterns exhibited by a molecule during mass analysis are characteristic of a specific species. However, EI does not impart exactly 70 eV to every molecule that is ionized, thus there are a range of energies imparted to formerly neutral molecules[12]. Ionization occurs when energized electrons are created and then interact with neutral species. The energy transfer between the energized electrons and analyte molecules is not

collision induced, rather the transfer occurs as a result of equilibration of a charge gradient[12]. Pure compounds exhibit a characteristic EI mass spectrum, therefore allowing a positive identification of a compound by mass spectrometry. In EI analysis of controlled substances, a positive confirmed identification is based upon an EI mass spectrum that contains the characteristic fragmentation pattern specific to the molecule in question. Figures 5 and 6 are samples of typical EI spectra. These EI fragmentation patterns can be used as the basis of a selected ion monitoring (SIM) - chromatographic profile for confirmation with EI-MS, according to several professional bodies such as the College of American Pathologists. However, a confirmatory scheme for ink is not as straightforward as for controlled substances. The ASTM E-1422-05 Standard Guide for Test Methods for Forensic Writing Ink Comparison does not include a procedure to confirm two ink samples are “identical or the same ink.” The batch-to-batch variation of inks, in general, only allows the following conclusions; the inks do not have a common origin (differentiation), the inks are of the same or similar formulations, see Table 2.

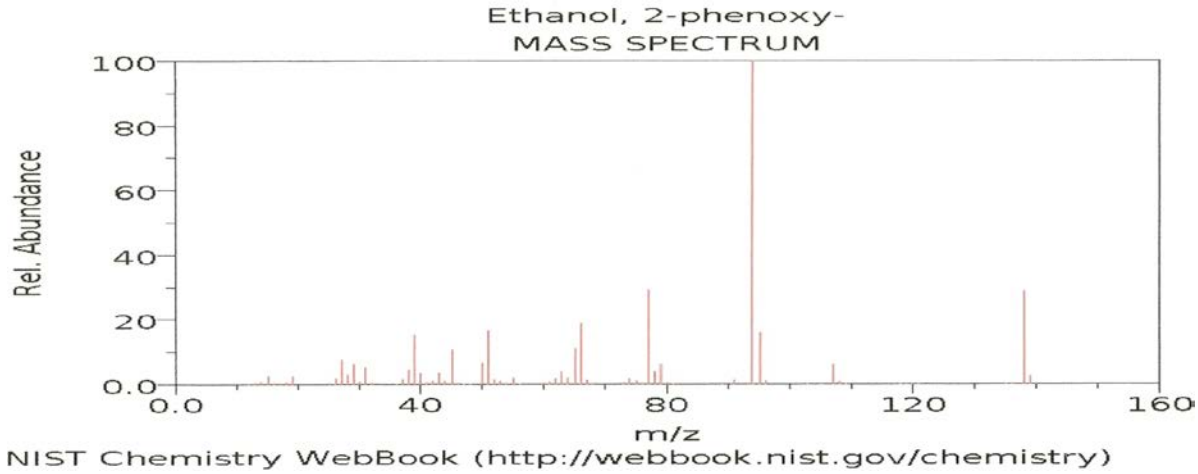

Figure 5: Electron ionization mass spectra of 2-phenoxyethanol (molecular mass 138.17)

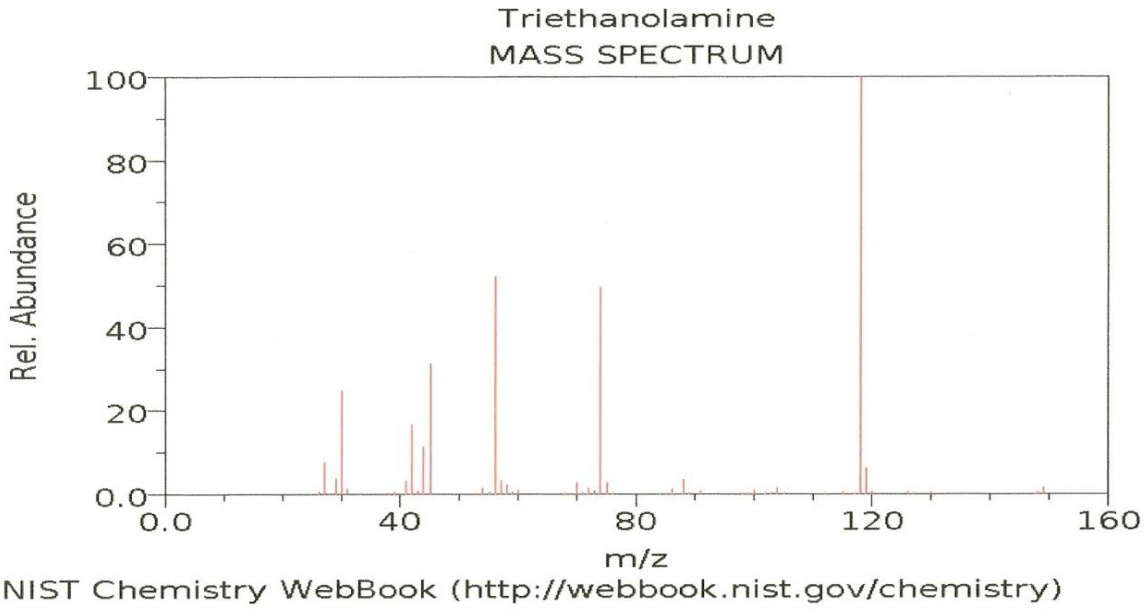

Figure 6: Electron ionization mass spectrum of triethanolamine (molecular mass 149.188)

GC-MS methods were used to identify ballpoint ink volatiles, specifically 2-phenoxyethanol EI spectra in Figure 5 which has been used to determine the time since deposition age of ink on paper[13]. The author of the standard guide surmised that the rate of evaporation of solvent for a heated sample would eventually equal a constant value. The age of the writing would be equal to the time required for the amount of 2-phenoxethanol, see figure 7, in an artificially aged sample, to equal the amount found in a sample of unknown age. Accounting for the loss of 2-phenoxethanol illustrates a common problem in ink analysis as it relates to questioned document analysis, the effects of aging and other factors on ink identification and classification.

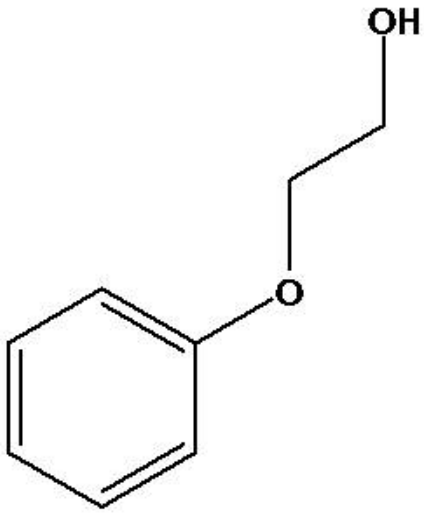

Figure 7: 2-Phenoxyethanol molecular structure

There are a large number of writing instruments available commercially with various ink formulations, each of which potentially contains an unknown variety of chemical species and compound classes that must be identified in order for a positive and confirmatory result. Ink, by its nature, is a complex mixture of ingredients blended to exhibit commercially desirable proper–ties. Positive identification of compounds with complex EI fragmentation patterns is a difficult task. Typically, a comparison to a known standard is the preferred method to conclusively identify an unknown sample. The National Institute for Standards and Technology (NIST) mass spectral database, a database of greater than 100,000 mass spectra, was generated using mainly EI methodologies [12]. GC-EI-MS methods have historically been the method of choice for the analysis of samples of unknown composition. However, the versatility of the liquid chromatography (LC) and liquid chromatography mass spectrometry (LC-MS) instrumentation are making their use in forensic trace evidence analysis indispensable and more commonplace.

Liquid Chromatography Mass Spectrometry (LC-MS) Analysis of Dyes

Liquid chromatography mass spectrometry (LC-MS) has been utilized in the analysis of organic molecules of all types as well as inorganic salts, and volatiles. An analytical column is used in LC analysis to provide chromatographic separation of a sample into its components by interactions with the stationary phase. After chromatographic separation is completed, the analytes must be ionized before mass analysis. There are two commonly used atmospheric pressure ionization methods utilized with LC-MS, electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI). Each method has advantages and limitations. APCI methods were not used in this research and will not be considered further. LC/ESI-MS methods have been adapted to some forensic analytical schemes, such as identification dyes from extracted from textile fibers or writing inks [2, 14, and 15]. A very broad range of analytes can be used with ESI-MS methods.

Electrospray Ionization (ESI)

Electrospray ionization is considered a "soft ionization" method, which results in little fragmentation of analyte molecules before mass analysis[16]. For instance, see Figure 5 for a sample of a typical ESI spectrum of 2-phenoxyethanol. ESI is an accepted method for analysis of polar, thermally labile, multiply charged, and non-volatile compounds. ESI mass spectra contain 'quasi-molecular ions' as opposed to molecular ions and fragment ions that are observed with EI spectra. The types of quasi-molecular ions generated by an ESI interface are typically the protonated molecular ion $[M+H]^+$, deprotonated molecular ion $[M-H]^-$, or an adduct ion such as $[M+Na]^+$[16]. ESI methods are subject to forming adducts of molecules with ions in solutions such as sodium or potassium[17]. Adduct formation can be exploited to detect some chemical

species, for example some glycols form adduct ions quite readily with sodium. One unique feature of ESI analysis is the formation and detection of multiply charged ions, where z in the mass to charge ratio (m/z) is greater than one[16, 18]. Some compounds exhibit multiple adduct forms for example, $[M+(n)Na]^{n+}$, which can form visually recognizable patterns. The mass spectral peaks associated with the compound are spaced 23 m/z units apart in the case of sodium[12, 18]. Adduct formation is not limited to sodium ions, they can also be formed by chloride ions or ionized solvent molecules, i.e. triethanolamine adduct $[149+Na]^{+}$= 172 m/z as seen in Figure 9.

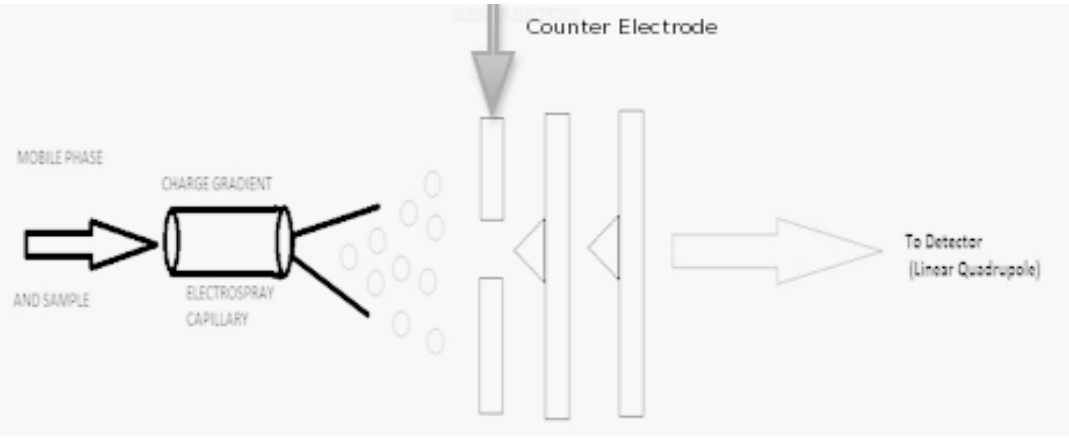

Figure 8: Electrospray ionization interface

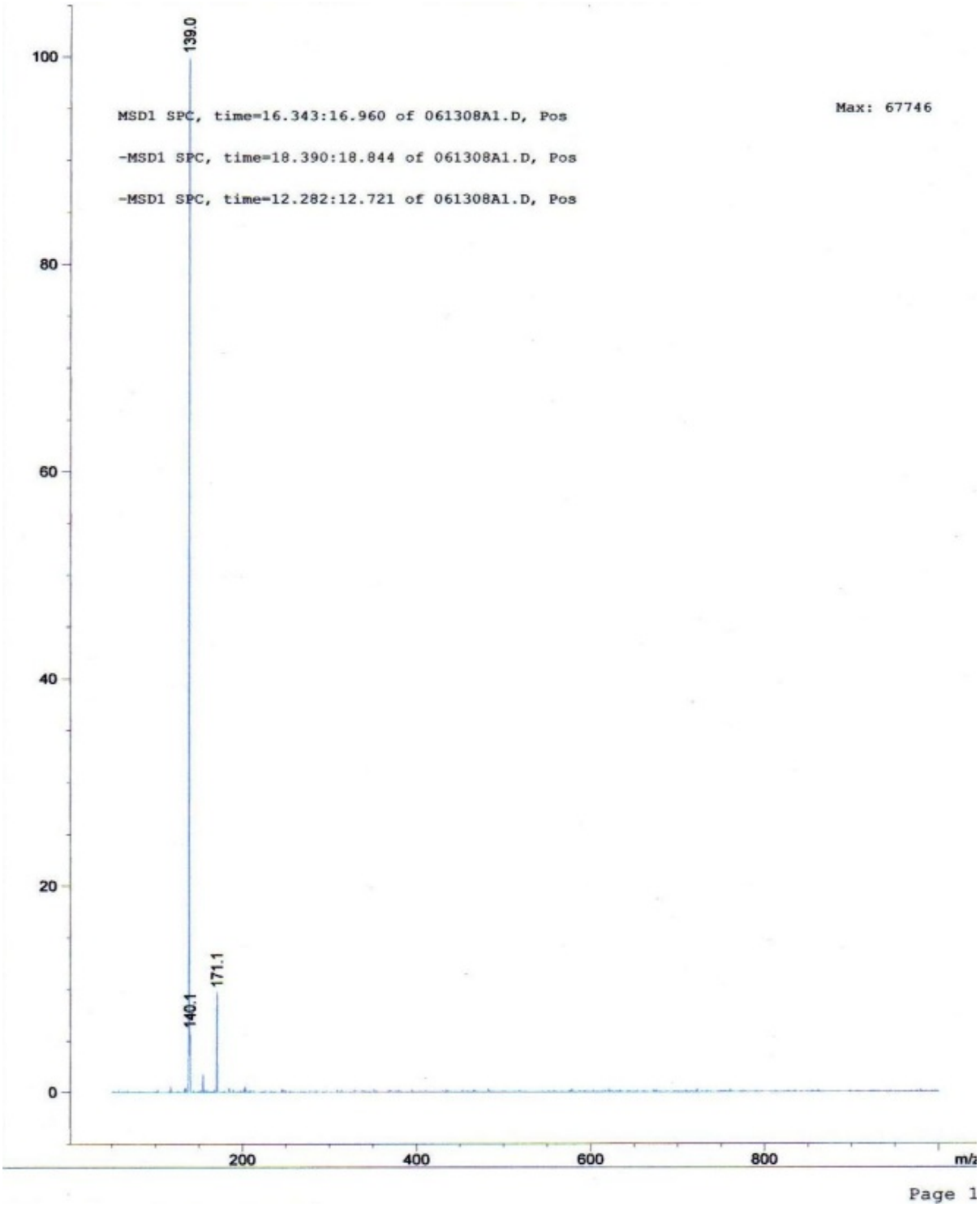

Figure 9: Electrospray ionization mass spectrum of 2-phenoxyethanol molecule [M+H]=139

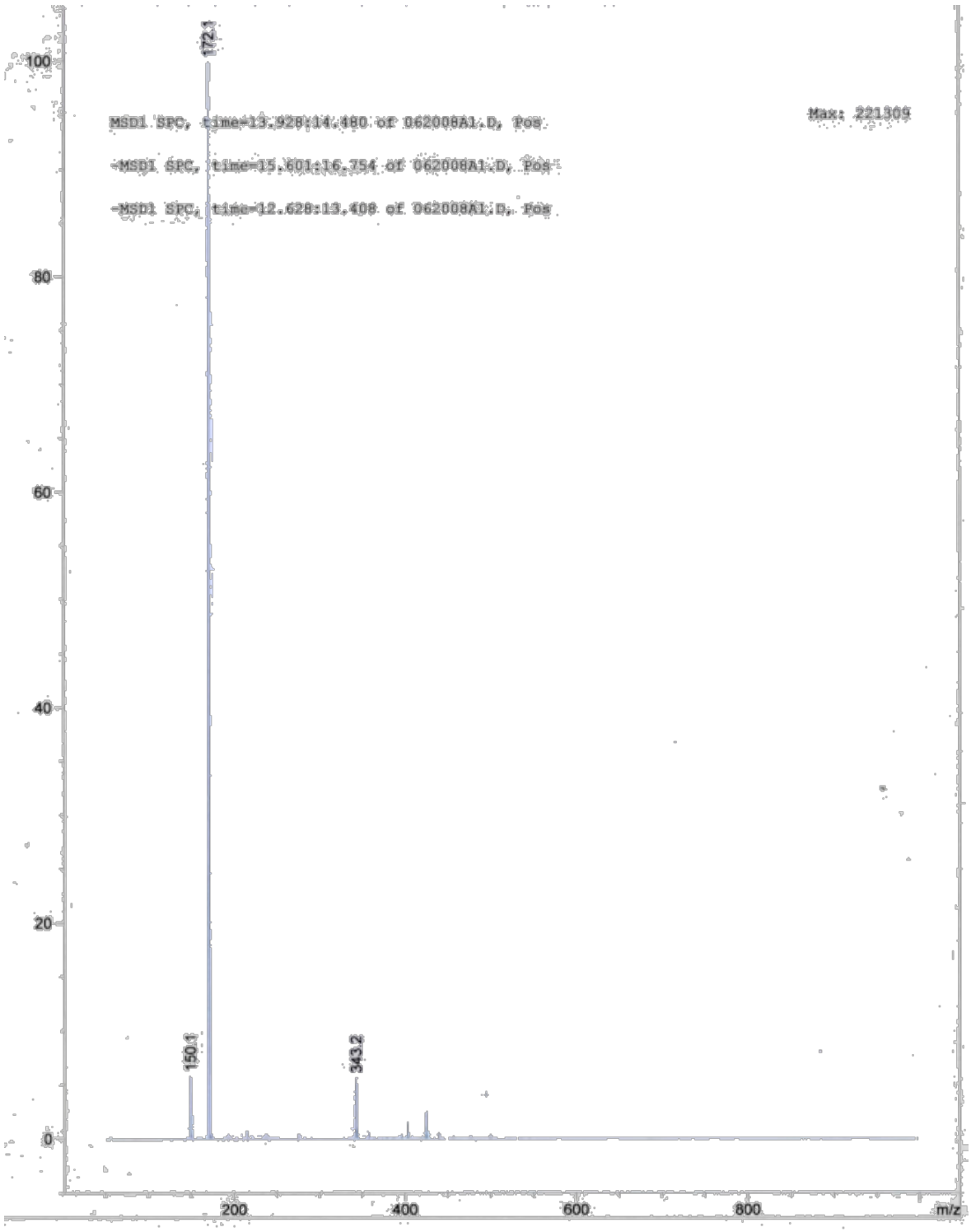

Figure 10: Electrospray ionization mass spectrum of triethanolamine [M+Na] = 172 m/z

The ESI process begins with the nebulized aerosol of charged droplets (see Figure 8). Charge is imparted to the mixture as it passes an electric field at the end the electrospray nebulizer as the liquid is dispersed. The introduction of heated helium drying gas volatilizes the solvent from the charged aerosol droplets, which results in smaller droplets of high analyte concentration. The charge of the liquid entering the nebulization chamber and the subsequent charged droplets depend on the analysis polarity, either positive or negative. The droplet gets smaller as more solvent is evaporated and carried away with unionized and neutral molecules (Figure 8). Charge density on the droplet surface eventually becomes so great that the surface tension of the droplet can no longer contain repulsive forces of the ions, and gas phase ions are generated (see Figure 8). These gas phase ions are led by gas flow and a potential gradient in a heated glass capillary to the mass analyzer. ESI allows the analysis of a large number of compounds across varying chemical classes; however, a pre-nebulization additive could be used in order to analyze samples that are not ionized by the dissolution process. The addition of an additive such as ammonium chloride or acetic acid can increase the effectiveness of the ionization process and elicit an increased instrumental response[19]. ESI interfaces allow the use of less complicated and less expensive mass analyzers and are commonly encountered with linear quadrupole type mass analyzers.

Linear Quadrupole Mass Analyzers

The use of ESI allows the detection of ions having single charged or those that are multiply charged can be introduced into the mass analyzer[12]. A quadrupole mass analyzer, used in this research, operates by separating ions of differing mass to charge ratio (m/z). This is accomplished in an electric field applied between the elements in the quadrupole (see Figure 11).

The electric field is varied in order to allow only ions of a particular m/z to pass through the mass analyzer and reach the detector, typically an electron multiplier. Ions that do not meet the criteria (the selected m/z) are deflected and do not reach the detector. A scan performed with EI mass analyzers allows many differing m/z ions to be detected. A scan for the m/z range of the mass analyzer is accomplished by varying the DC voltage to allow ions from a set m/z ratio to pass thought the quadrupole to the detector.

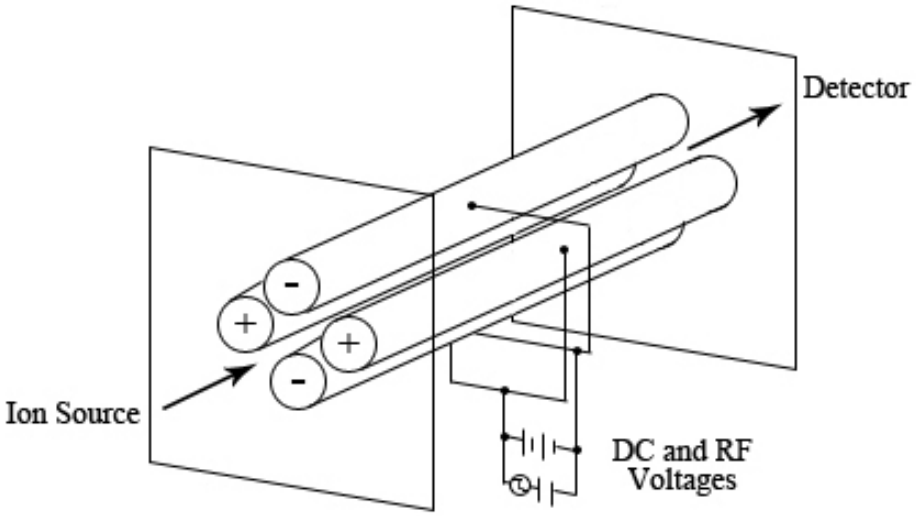

Figure 11: Diagram of quadrupole mass analyzer (reprinted with permission)

Quadrupole instruments are composed of an introduction system for ions created in the source, typically a series of skimmers and a capillary. The quadrupole itself is four rods that are oriented parallel to each other. This creates a channel in the center of the four rods that is the path that ions of the selected mass (m/z ratio) will pass through, to the mass analyzer (Figure 11). The application of DC and AC voltages to the rods allows the instrument to filter all but one mass to charge value at a time.

LC-MS ESI Direct Infusion Analysis of Inks and Dyes

Several methods have been described for ESI-MS/MS identification of dyes extracted from textile fibers and ink dyes in instruments where the analytical column is bypassed and the mobile phase and sample are directly pumped to the ionization interface [2, 20]. This method is known as direct infusion ESI-MS. With direct infusion method, no chromatographic separation of compounds is achieved. However, with use of the proper ESI polarity, mobile phase additive, and fragmentor voltage, a mass spectrum containing many of the components in an ink sample can be obtained in a single scan. The method may allow an individual ink sample to form a unique characteristic signature or 'fingerprint.' This fingerprint can be compared to other inks, and a determination of similarity or difference can be assessed. The signature would include the colorants, vehicles, and other ink constituents. A LC/ESI-MS method compatible with BP, RB, and GP inks and based on uniqueness of mass spectral information is presented in this thesis[2].

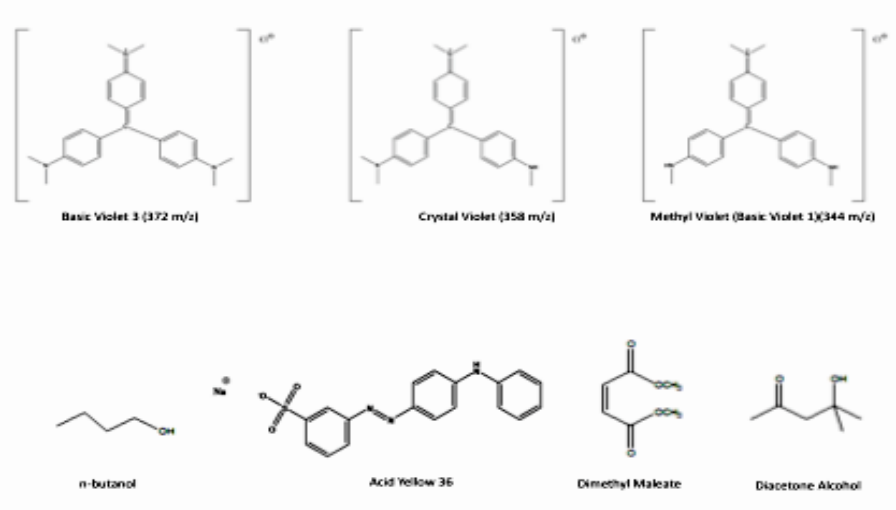

Figure 12: Molecular structures of selected ink components

Challenges in Ink Analysis

Among the many challenges of ink analysis is the effect of age upon analytical results. Immediately after an ink mark is deposited on paper, the volatile solvents act to disperse the colorants in an even coating on the paper. As time passes, the solvents will eventually no longer remain as part of the ink mixture on paper. It will be below detectable limits at a point in time determined by, but not limited to, storage temperature, vapor pressure, humidity, light exposure, the physical and chemical properties of the mixture of chemicals in the ink, and the length of time since initial deposition. The bulk (98%) of BP solvent is volatilized and dispersed into the surroundings within the first two minutes post deposition on paper[21]. Any method used to detect residual solvents must be sensitive enough to detect these solvents at roughly 1% of total composition with background and paper interference. One form of age determination known as bracketing, fixes the earliest date an ink formulation was available, which can be compared to dates on the document. Others have suggested an aging/dating method that would not rely on limited trace quantities of volatile components, but rather focusing on a method based on analysis of the sum of extractable ink components.

One of these aging methods is the determination of degradation of hexamethylpara-rosanaline or basic violet 3(BV3). BV3 is found in the majority of black and some blue ballpoint inks as the main synthetic dye. Basic Violet 3 is a triphenylmethane derived dye containing six methyl groups which are able to undergo demethylations (methyl replacement by hydrogen) when exposed to sources of light, in visible or ultraviolet wavelength range[22]. The demethylations occur through a reaction induced by interactions of the dye with photons of incident light[23]. With mass spectrometric methods, the demethylations of BV3 can be

visualized in the ESI mass spectrum as an ion 372 m/z, with a smaller peak at 358 m/z, and sometimes a peak located at 344 m/z.

However, BV3 as shown in Figure 12 is rarely encountered in a pure form, since there is nearly always some included basic violet 1 (358 m/z). BV1 is also a degradation product of the hexamethalyted molecule, and as such, a BV3 sample that has been exposed to light will always contain some BV1, including the BV3 found in pen inks. The ratios of BV3/CV/BV1from methanolic extracts of a series of blue BP ink marks stored in controlled conditions and those subjected to "various places and conditions" [24]. The CV/MV/TPR ratios of the control group after two years of exposure at room temperature through glass to ambient solar radiation, the shift towards the doubled demethylated CV form was noted. As a result, there is less of the TPR form detected when analyzed with High pressure liquid chromatography-UV diode array detector (HPLC-UV/DAD), to the extent that the treated samples could not be distinguished from the control samples[24]. Some recent LC-MS ink analysis methods call for tandem mass spectrometry that require very new or complex ionization techniques. Sophisticated instrumentation such as this invariably carries a price tag out for reach of many crime labs operating with reduced budgets. Some other analytical instrumental methods like capillary electrophoresis have been adapted to ink analysis.

Other Ink Analysis Methods

Some previously described methods of ink analysis used thin layer chromatographic analysis (TLC). TLC has been used to separate dye components of BP inks, dyes from each other, as well as some vehicle components from other ink types. A series of other destructive tests provide the analyst with more information about the questioned ink. Besides wet chemical

methods, there are newer available hyphenated instrumental analysis methods including ICP-MS, CE-MS, and some tandem MS methods have been adapted for ink or dye analysis.

Thin Layer Chromatography (TLC) Analysis

TLC is a standard ink analysis technique used in a significant number of published ink analysis methods, since the equipment is inexpensive compared to the cost of a MS instrument. In addition, TLC typically requires little operator training. However, one criticism of TLC analysis is the consumption of ink lines from the paper. With a blunted 0.5mm-2.0mm biopsy needle, several (5 to 10) punch sections may be required for one TLC spot sample. More than one sample per ink maybe needed in order to differentiate exhibits or to make comparisons to other inks. Libraries of TLC chromatograms are regularly used for the basis of comparison of known standard ink and unknown ink samples. The discriminating power of TLC methods can be increased with densitometric and spectrophotometric analysis [9]. The use of chemical spot tests can also be useful in the total analysis of questioned ink samples and provide class differentiation.

Chemical Spot Tests

The hypochlorite spot test is one of the quicker methods for an analyst to determine the class of a questioned ink. The test is performed by treating a portion of the sample that has been spotted on a TLC plate with sodium hypochlorite. If any movement of the ink spot is noticed when a 10% sodium hypochlorite (bleach) solution is added, the sample is not a gel ink. Since gel inks contain insoluble pigments such as carbon black that is not subject to chemical bleaching [3]. Ballpoint inks will run when organic solvent is applied, for example pyridine. [10]

Brew, Hagen, and Egan of the U.S. Federal Bureau of Investigation Laboratory, used capillary electrophoresis (CE) with ultraviolet visible detection for the analysis of both black and blue BP ink formulations. The CE/UV methods suffer the same drawbacks as UV methods when the chromatographic separation was lacking [25]. A matrix-assisted laser desorption/ionization (MALDI) method with direct sampling from the questioned document has been reported [26]. The MALDI method can detect multiply charged dyes without additives; however, for detection, the singly charged dye 2-(4-hydroxyphenylazo)benzoic acid (HABA) is added to the MALDI matrix and diammonium hydrogen citrate (DAHC) is applied to the questioned document for detection with MALDI-MS [26]. Instrumental requirements of MALDI analysis include newer time-of-flight mass spectrometers having a high sensitivity and short laser ionization times.

Fluorescent compounds or rare earth elements were added to ink formulations by some manufactures from 1970 to 1994 at the request of the United States Secret Service [27]. The addition of these chemicals, referred to as “taggants,” served to help identify and individualize inks samples provided they were included in the tagging program. However, less than 40% of ink manufacturers participate and the program was discontinued in 1994 [27]. Inductively coupled plasma mass spectrometry can be used to detect rare earth elements sometimes used as taggants [27]

CHAPTER TWO: EXPERIMENTAL

Sample Preparation

All ink samples were purchased in the Orlando, Florida area. The set of pens were used on Quick Copy Xerographic DP - White paper, which was used with all samples except the investigation into effects of paper on ink analysis. Two types of samples were studied; paper strip and fiber collections. The strip samples consisted of paper strips 1/8 inch wide and 3 inches long coated on one side with a large amount of ink deposited on its surface. Fiber samples were taken by removing a small amount of ink-coated fibers from a written mark, as in Figure 13, by using forceps and a metallic probe under a stereomicroscope. Several of the fibers were placed at the closed end of a glass melting point capillary tube and 20 μL of solvent was added with a Hamilton 100 μL blunt ended liquid chromatography injection syringe. Inks used in all phases of this research are listed in Table 2, Chapter 1. They are listed by the assigned pen number, pen name designation, and ink type class.

Figure 13: Close up of a sample after removing enough fibers to perform an extraction

Instrumental Methods

Direct Infusion Electrospray Ionization Mass Spectrometry (ESI-MS) Analysis

An Agilent 1100 series linear quadrupole mass analyzer was used for ESI-MS analysis. This system was configured to by-pass the chromatography column and uses the instrument's binary pump to force mobile phase toward the ESI interface. A Rheodyne manual injection port with a 5-µL sample loop was used to introduce a sample into solvent flow. A syringe pump was used to infuse a pre-nebulization additive. The polarity, additive concentration and applied voltage were varied to optimize a method for mass spectral characterization of ink on paper samples. Three analytical methods were developed; (Method 1) +120V with 4.1% (vol/vol) trace grade glacial acetic acid in methanol, (Method 2) +0V with 0.00041% (wt/vol) sodium acetate in Methanol, and (Method 3) -120V with 4.1% (wt/vol) triethylamine in Methanol.

Instrumental Parameters

The suggested extract volume per ink sample is 20 µL, however a 5-µL sample loop was used for this research. The sample loop was overfilled in order to limit the variation of injection volume. Rheodyne Technical Note Number 5, indicates that volumetric precision of the method of injecting a two- to five-fold volume excess into the manual injector port gives an RSD of 0.2% for volume injected. Sample extracts were introduced into the ESI interface using a Rheodyne 8125 manual sample injector with a 5-µL sample loop. Mobile phase was supplied by an Agilent LC binary pump at a flow rate of 200 mL/min. Before introduction into the ESI interface, a mobile phase additive was also infused with the mobile phase from a screw-drive type syringe pump introduction system with a rate of 5.0 ML/min. Once the sample was in the mobile phase flow it is moved into the ESI interface for subsequent mass analysis. The ESI

interface was kept at 350 °C, with a 12.1 mL/min flow of nitrogen drying gas at 30 psig. The fragmentor applied voltage was adjusted from -120V to +120V in order to determine which setting provided the least fragmentation and the best response for all three ink classes. The mass filter was set to scan from 50 to 1000 Daltons.

Extraction Optimization

The best universal solvent for extraction of ballpoint, rollerball, and gel inks was determined by test extractions from ink-coated paper strips. Initially, pens 1–6 and 10-22 on Table 2, page 9, of the initial 33 pens were extracted and placed into either ethanol, methanol, or benzyl alcohol, where they were allowed to sit 30 minutes at room temperature in a flame sealed glass melting point capillary. After 30 minutes the samples with methanol exhibited color change with more samples than ethanol, or benzyl alcohol. While the dyes contained in ballpoint and some rollerball inks are quite soluble in alcohols, gel ink colorants are insoluble and required additional consideration during the method development phase. Sonication of fibers and solvent for times of zero and thirty minutes showed no effect on ions observed in the sample's ESI-MS spectrum. A series of three to four-inch long marks from inks that did not extract with methanol, ethanol, or benzyl alcohol were deposited on a Whatman #2 'qualitative circles' filter paper and 20 μL of various solvents were spotted on the ink lines. Each of the solvents was observed after ten minutes for possible movement of ink color. Solvents reportedly used in TLC analysis of inks were examined for possible use for extraction of gel ink (pens 13, 14, 15, 17, 18, 20, 22) components, e.g. hypochlorite, ethyl acetate, pyridine, glacial acetic acid, THF, 10% ammonium hydroxide solution, isopropanol, acetonitrile, DMSO, and DMF. The inks were determined to be

soluble for this group of pens [1]. Very pure methanol such as LC-MS grades are reasonably in cost and can be used across all three ink classes discussed in this research.

Direct Infusion ESI-MS Optimization

Spectral Subtraction to Yield "Pure Ink" Spectrum

Samples were analyzed using a subtraction method. A series of samples were injected in the following order, a solvent blank (syringe blank), "paper blank", solvent blank, and methanol extract of the ink sample. The series of injections were collectively referred to as an 'analytical run' and is the basis for direct comparison of ink spectra to one another. The ion elution profile shown below was typical of ballpoint ink-on-paper methanol extract, as depicted in figure 14.

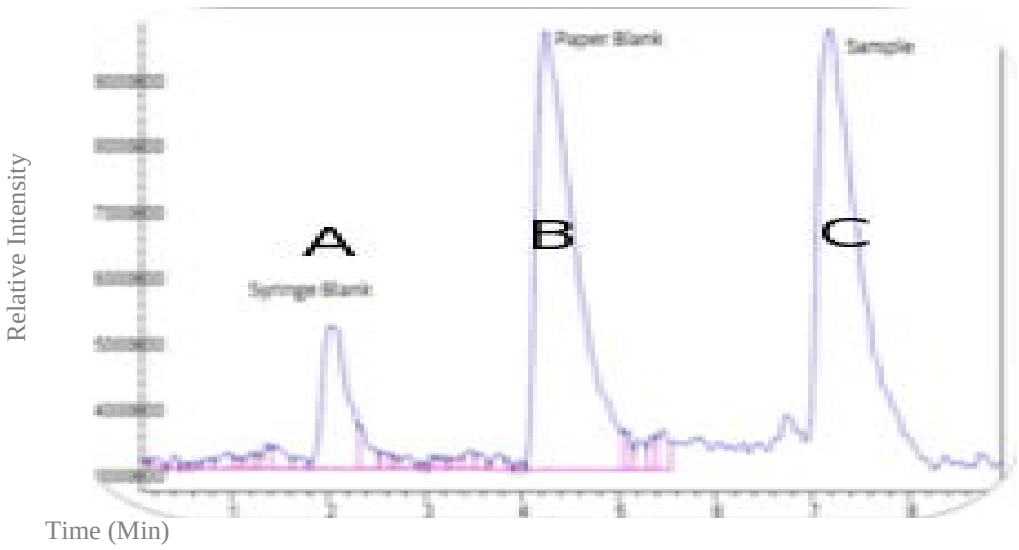

Figure 14: Chromatogram showing elements of the subtraction method (analytical run)

Within the Agilent Chemstation software, the peak corresponding to the ink-on-paper sample was selected (4 to 5 min) in figure 15. The resultant mass spectrum contains background, mobile phase, sample, paper, and "pure ink spectrum" components. The mass spectrum

corresponding to the peak at 1.8 minutes was subtracted from the sample mass spectrum resulting in the spectrum shown in figure 15B and then the solvent blanks were subtracted (figure 15A). The remaining spectrum (paper blank, background, and syringe blank subtracted) is the "pure" spectrum of the ink sample on paper (see Figure 15D). Once this spectrum is normalized, it is ready for comparison to other ink spectra.

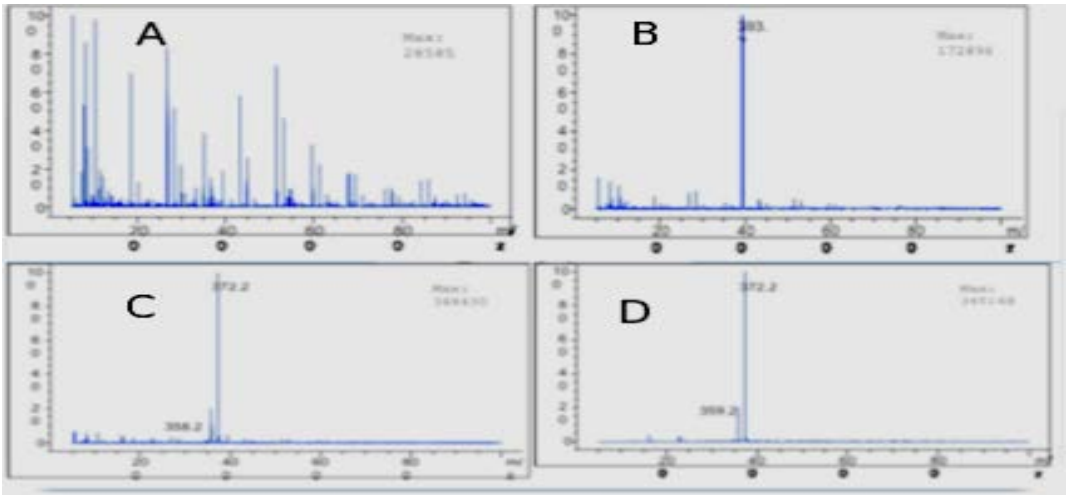

Figure 15: Subtraction method

Ink Volatiles Persistence Study

A representative pen was chosen from each of the three ink classes for a study of drying time vs. extractable volatiles. With each of pens 2, 13, and 18, lines were drawn on clean white copy paper and one sample was extracted before it was allowed to completely dry, one sample was allowed to dry to touch (30 minute dry time), samples were also allowed to dry for 1 hour and 24 hours. The samples were extracted and analyzed with a sodium acetate additive in methanol at a +0 V applied fragmentor voltage, the best method for the volatile components.

Both strip and fiber samples are used in the assessment of the effect of drying time on extractable volatiles.

Effect of Paper on Extraction

The effect of paper type on a deposited ink sample's ESI mass spectrum was examined by analyzing the ink from one pen on various paper substrates. The ink was deposited on to twenty differing colors and types of papers and then extracted after a 30 minute drying period. The mass spectrum of the recovered ink was examined after subtraction of the syringe/solvent blank and the paper blank from the ink plus paper matrix.

Dye and Vehicle Standards

Dye and vehicle standards were made in filtered LC-MS grade methanol, and subsequently analyzed with the direct infusion method (see Tables 3, 4, and 5). Standards were analyzed at +120V with no additive, initially to determine if the molecular ion could be observed in the standards mass spectrum. The observed ions were recorded for comparison to results from inks analyzed with the method.

Table 3: Positive dye standards used with Method 1

Dye Name	CAS Number	Concentration Used	Formula Weight
Solvent Black 3	4197-25-5	5 ppm	456.55
Basic Red 1	989-38-8	6.5 ppm	479.02
Disperse Orange 25	31482-56-1	5 ppm	323.35
Basic Violet 1	8004-87-3	6.5 ppm	456.17
Solvent Orange 3	495-54-5	5 ppm	212.28
Basic Violet 10	81-88-9	6.5 ppm	479.02
Solvent Red 49	509-34-2	5 ppm	442.55
Basic Blue 7	2390-60-5	5 ppm	514.16
Basic Violet 3	548-62-9	6.5 ppm	407.92

Table 4: Negative dye standards used with Method 3

Dye Name	CAS Number	Concentration used	Formula Weight
Acid Red 51	568-63-8	5 ppm	879.87
Acid Red 87	17372-42-1	5 ppm	691.88
Solvent Orange 3	495-54-5	5 ppm	212.28
Acid Yellow 3	8004-92-0	5 ppm	477.05
Acid Yellow 36	587-98-4	5 ppm	375.38
Acid Yellow 23	1943-21-0	5 ppm	534.37
Acid Yellow 73	518-47-8	5 ppm	376.28
Acid Green 1	19381-50-1	5 ppm	878.47
Acid Blue 9	3844-45-9	5 ppm	792.86
Acid Blue 92	3861-73-2	5 ppm	695.59
Solvent Blue 38	1328-51-4	5 ppm	734.65

Table 5: Vehicle standards used with Method 2

Vehicle Name	CAS Number	Concentration used	Formula Weight
Glycerol	56-81-5	50 ppm	92.09
Dimethyl maleate	624-48-6	50 ppm	144.13
Diethyl phthalate	84-66-2	50 ppm	222.24
2-Phenoxy ethanol	122-99-6	50 ppm	138.16
Ethylene glycol	107-21-1	50 ppm	62.07
1-H Benzotriazole	95-14-7	50 ppm	119.13
Diacetone alcohol	123-42-2	50 ppm	116.16
Diethanolamine	111-42-2	50 ppm	105.14
Dioctyl phthalate	117-81-7	50 ppm	390.56
Benzyl alcohol	100-51-6	600 ppm	108.14
n-Butanol	71-36-3	No dilution	74.12

In order to optimize the analytical method for applicability to all three ink classes, a set of positive, negative or vehicle standards were injected, together with standards from each ink class at one voltage setting and one additive concentration. The applied fragmentor voltage was set at +0V, +60V, or +120V for the positive ion forming dyes in order to determine the acetic acid additive and fragmentor voltage settings that provided the best instrumental response. For the anion forming dyes, triethylamine was used and the voltage settings were -0V, -60V, and -120V.

Sodium acetate additive was used for ink vehicle detection, and was observed at +0V, +60, and +120V to determine the best setting to insure minimal molecular fragmentation. Once the optimal conditions were determined, limits of detection were calculated from calibration curves of standards at the optimized voltage, additive, and polarity settings.

Photochemical Experiments

The ballpoint pen ink used for photochemical exposure experiments was selected from the set of BP ink examined because it had the lowest average intensity of the 358 m/z ion relative to the 372 m/z ion intensity. The lower the initial amount of 358 m/z present in ink 1, as compared to all of the initial six ballpoint inks, allows for better visualization of the photolysis via mass spectrometric techniques. Ink from pen 1 was used to create several five inch long pen marks with the aid of a ruler. A portion of the paper, on a cardboard support, was covered with aluminum foil to provide unexposed control samples for spectral comparison. The paper was stapled to the cardboard in each of the four corners. Once the foil was securely fastened, the sample was suspended three to four inches below the bottom of the Rayonette Photoreactor (RPR 350). The sample was exposed to a UV light for a given a one, two, three, four, and five hour time periods with use of a multifunctional timer. The ink was sampled in triplicate at each time, extracted with solvent and the capillary tube flame-sealed.

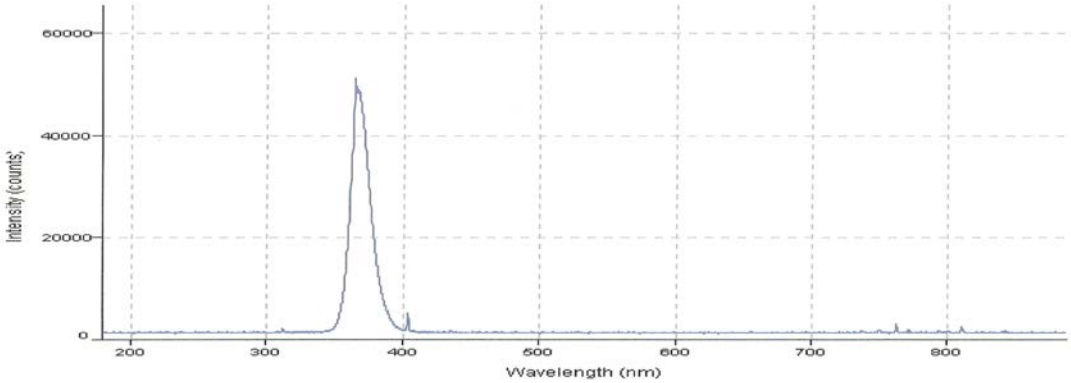

Figure 16: RPR photoreactor spectral output

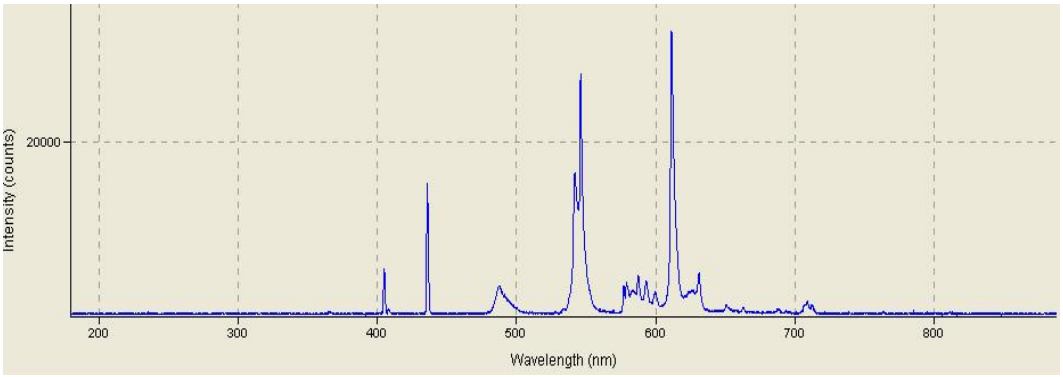

Figure 17: Spectral output profile for GE Helical light bulb at a distance of 12 inches from the source

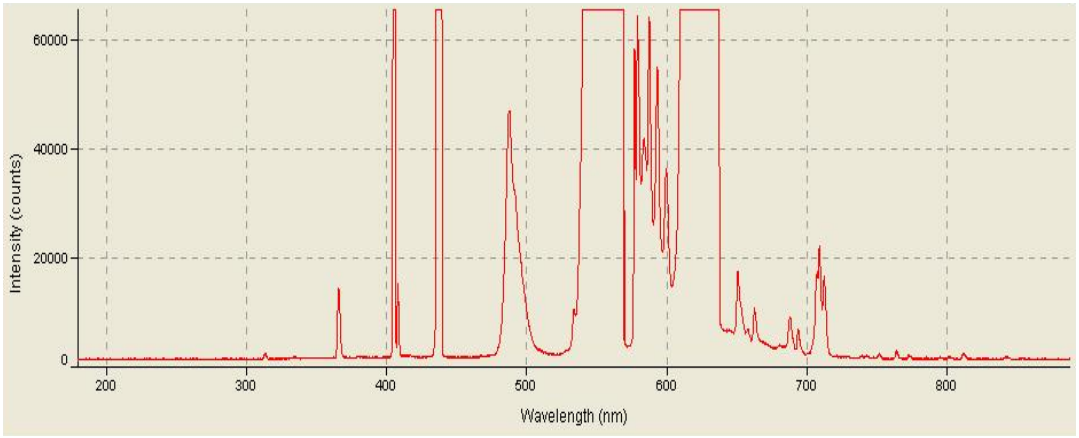

Figure 18: Spectral output for GE Helical light bulb at a distance of 7 inches

The extent of degradation of the ink was determined based on the relative peak area (RPA) of m/z 372, 358, 344, 330, 316, 302, and 288 were as used by Weyermann to evaluate the degradation process with MALDI analysis methods. With ESI analysis, the presence of an ion at 372 m/z is indicative of BV3, however even "commercially pure" BV3 samples contain some of the 358 m/z ion and 344 m/z ion as impurities. When one attempts to determine the extent or rate of BV3 degradation, the sequential loss of methyl fragments to yield ions of 358, 344, 330, 316, 302, and 288 m/z ions.

The extracted ion peak area of 372 m/z ion from the sample divided by the total area of degradation products from 358 to 288 m/z values were used to, quantify the extent of degradation [22]. The degradation times for BP inks were determined using direct injection ESI-MS methodologies. Samples were extracted and analyzed with the direct infusion ESI-method. The area ratios of all of the products were compared after exposure to a light source.

Light Exposure Effects

Samples of BP ink are known to decompose upon exposure to light. Pen 1 was used to study the degradation detectable with the ESI-MS method. Samples were exposed to light sources from seconds to several days and photobleaching effects and exposure related degradation products to observed. Light sources used were a GE Helical desk lamp with an energy efficient standard light bulb, and a RPR3500 Rayonette UV photochemical reactor.

Potassium Ferrioxalate Actinometry

The quantum yield of the potassium ferrioxalate (PF) actinometer has been extensively studied, and has been determined for the absorbance profile range (250-509 nm). This method is based on the conversion of ferric ion to ferrous ion by light. The ferrous ion concentration is

indirectly determined by absorption of the ferrous- (1,10-phenanthroline) complex at 510 nm. The resulting complexed solution's absorbance at 510 nm was measured with an Ocean Optics USB200 UV-Visible spectrophotometer.

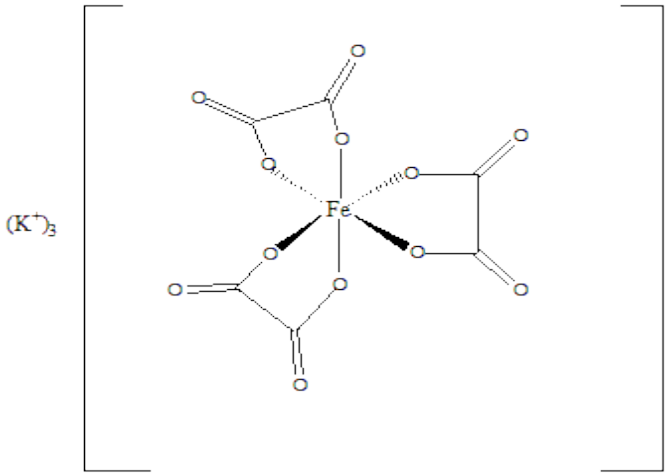

Figure 19: Potassium ferrioxalate ion

Potassium ferrioxalate ion, in Figure 19, was synthesized from three volumes of 1.5 M potassium oxalate and one volume of 1.0 M ferric chloride. The resulting green solid was recrystallized three times for purification as per the IUPAC actinometry guide[8]. The crystals were vacuum filtered, dried, and finally weighed. A quartz cuvette was charged with 3 mL of 1.5 M ferrioxalate solution and exposed to UV light from the Rayonette photochemical reactor for 30 seconds, 1, 2 and 4 minute exposures with aid of a countdown timer. After exposure, 1 mL of PF solution was added to a 10mL volumetric flask, along with buffer and 1,10-phenanthroline solution. Red darkroom safety light was used to insure that actinometer would not be exposed to any UV light until it is placed in the photo reactor cell and the timer is engaged. After one hour of complexation, the absorbance of the complex was recorded at 510 nm with a UV-visible

spectrometer. The number of photons per unit time were calculated from the actinometry experiment, and compared to the UV exposed ink samples. A curve was generated from the absorbance of the complex at 510 nm and the photon flux was compared to that of the sun.

Method Validation of Direct Infusion ESI-MS Ink Analysis

Analysis of Triplicate Run Data

Triplicate analyses of inks from pens 1-33 were conducted using all three ESI-MS methods (+120V with acetic acid, +0V with sodium acetate, and -120V with triethanolamine) to compare previously collected ink data for sample-to-sample variations. All samples were the fiber type and were extracted into LC-MS grade Optima methanol after 30 minute drying time.

Same Manufacturer Multipack Pen Analysis

A set of pens from the same manufacturer, having the same class with different colors, were examined by direct infusion ESI-MS and compared to the black ink data. When black ink is dissolved in solvent, it can take on a dark purple appearance during extraction. Several colors of ink may contain basic violet 1 and 3, in a lesser concentration than in black ballpoint inks, which may make identification of a sample more difficult. The typical ESI-MS spectrum of a gel pen contains vehicle components, which could be the same in all ink colors from a same manufacturer. Triplicate analysis of pen sets containing purple, black, and blue inks was compared to the black ink data from ESI-MS method validation experiments. Table 6, shows manufacturer, ink class, name of pen, and the colors of ink used for direct infusion ESI-MS methods. Two samples were taken from four-color pens that were collected at the 2009 American Academy of Forensic Sciences Conference in Denver, CO. These pens were given out as gifts to attendees and were only labeled with advertisements. The four-color pens contained

black, blue, red, and green inks of which only the black and blue inks were examined in this research (see Table 6).

Table 6: Colored Pens

Manufacturer	Class	Name	Colors analyzed
Pentel	BP	Ola	Black, Blue, Purple
Zebra	BP	Z-grip	Black, Blue, Purple
Pilot	GP	G-2 Gel	Black, Purple
Signo	GP	207	Black, Blue, Purple
Pentel	BP	R.S.V.P	Black, Grey, Blue, Purple
Bic	BP	4-color	Black, Blue
Unknown	BP	4-color	Black, Blue
Unknown	BP	4-color	Black, Blue

Intersecting and Overlapping Ink Strokes (Deposition Order Determination)

Direct Infusion LC-MS

An addition was created on white xerographic paper, in the form of “$30.00” (Figure 20) on a simulated check. This was written with pen 3 and allowed to dry to touch, or 30 minutes, and then changed to “$80.00” (Figure 21) using pen 5, with the addition of writing to alter the original writing. When the samples were created, care was taken to insure an overlapping of the two inks in order to determine if the presence of both inks could be detected. Ink was sampled in areas that contained only ink 3, only ink 5, and overlapping areas that contained both inks (see Figure 19). After extraction and direct infusion ESI-MS analysis the resulting data can be compared to both ink 3 and ink 5 for uniqueness and mass spectra contributions from both inks.

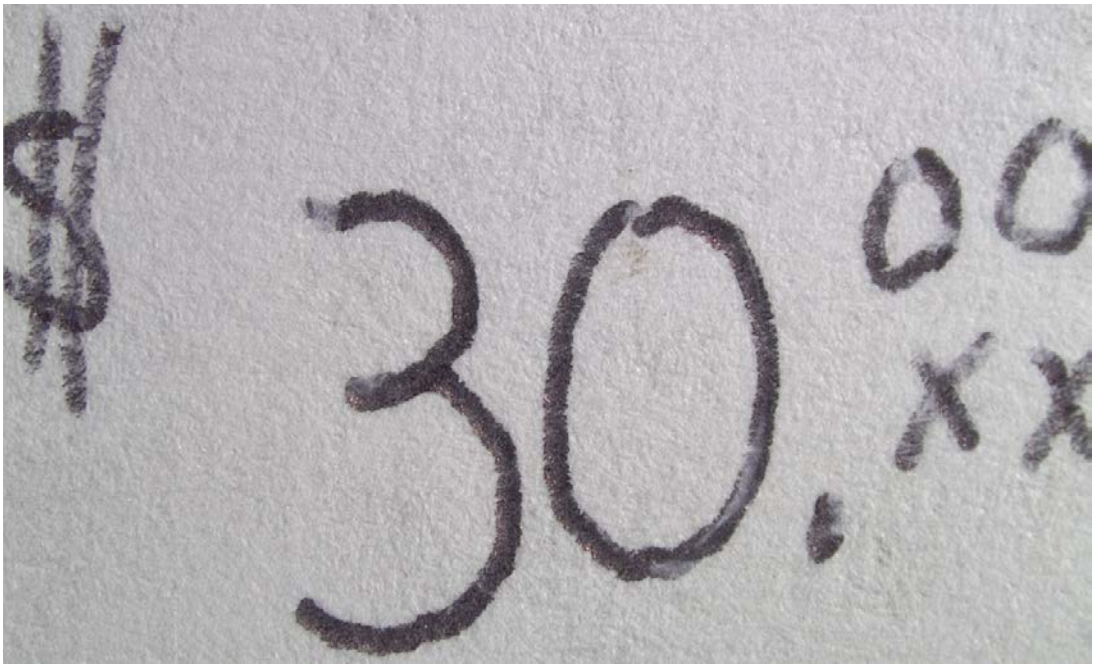

Figure 20: Pen 3 ink, original depicts an unaltered original writing of the number 3

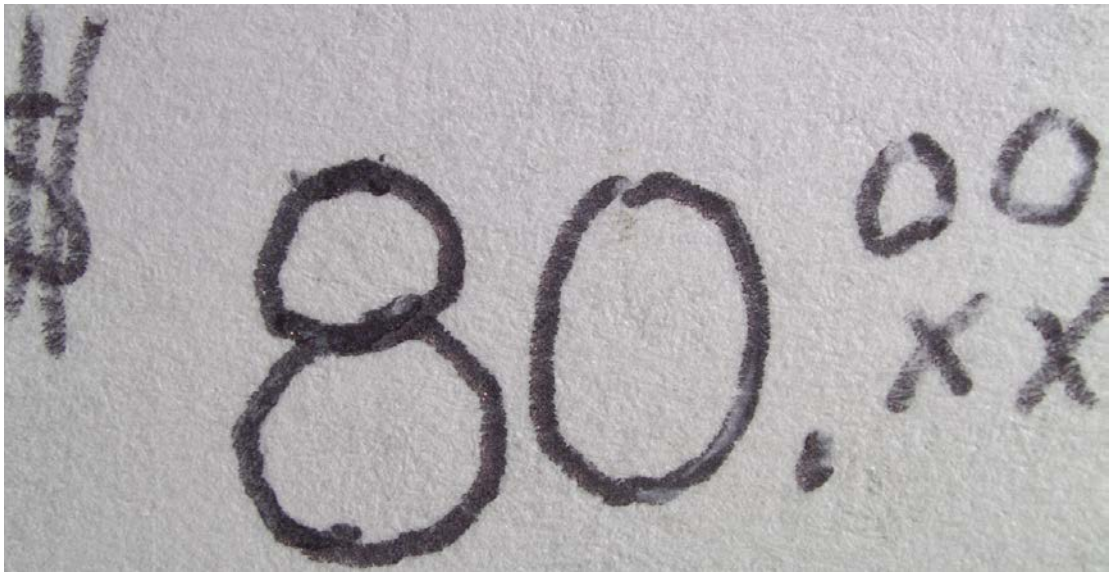

Figure 21: Pen 5 ink writing over written to change the value of figure 20 from a number 3 to a number 8

Figure 22: After sampling, showing the sampling of overlapping ink from pen 3 and pen 5

CHAPTER THREE: RESULTS

Direct Infusion Electrospray Ionization Mass Spectrometry (ESI-MS) Analysis

Extraction Optimization

The best choice of solvent to extract the largest number of the subset of pens 1-22 was found to be methanol. Pens 7, 8, and 9 were omitted from this study, since the inks were either felt-tipped pens or erasable ink pens. Methanol exhibited visible extraction of 9 of the subset of 19 (six ballpoint, seven gel, and six rollerball) inks. Absolute ethanol, benzyl alcohol, acetonitrile, deionized water, and mixed solvent systems only exhibited visible extraction of less than seven inks, leaving methanol as the best choice solvent for all three ink classes used in the research. Fisher Optima LC-MS grade cut methanol was used exclusively after two other grades of methanol exhibited increased sodium adduct formation in the positive mode at 120 V. Sodium adduct formation was not observed with LC-MS grade Optima methanol. The extraction solvent and mobile phase in all ESI-MS methods described in this research are 100% LC-MS grade Optima methanol. The use of methanol as the mobile phase and extraction solvent impedes the formation of polymers once a sample is extracted.

Direct Infusion ESI-MS Optimization

Method 1 (+120V)

The additive used with Method 1, 4.1% TraceMetal grade glacial acetic acid in filtered methanol, was introduced at a flow rate of 300 uL/min. The standard used to tune this mode was Basic Violet 3, which exhibits a large m/z 372 and a much smaller m/z 358 peak in its mass spectrum. Basic Violet 3 does not require an additive for ionization and detection in the mass spectrometer. Some potential ink ingredients include molecules that need an additive to assist in

ionization, such as Disperse Orange 25. These molecules typically exhibit an $[M+H]^+$ ion using Method 1, protonation due to the proton being liberated from acetic acid. See Table 7 for some ions observed with Method 1 for analysis. Figure 23 shows calibration data using Method 1 as the analytical mode for Basic Violet 3. The standard solutions of BV3 verified for concentrations via an inline Agilent UV-Vis detector and Ocean Optics USB UV-Vis system. The BV3 standard solutions were then used with direct infusion ESI Method 1 for comparison with another method based on a different scientific principal. An estimate of the amount of dye extracted from a particular sample is possible. The calibration using BV3 and Method 1 is relative since it depends on sample size and ink type.

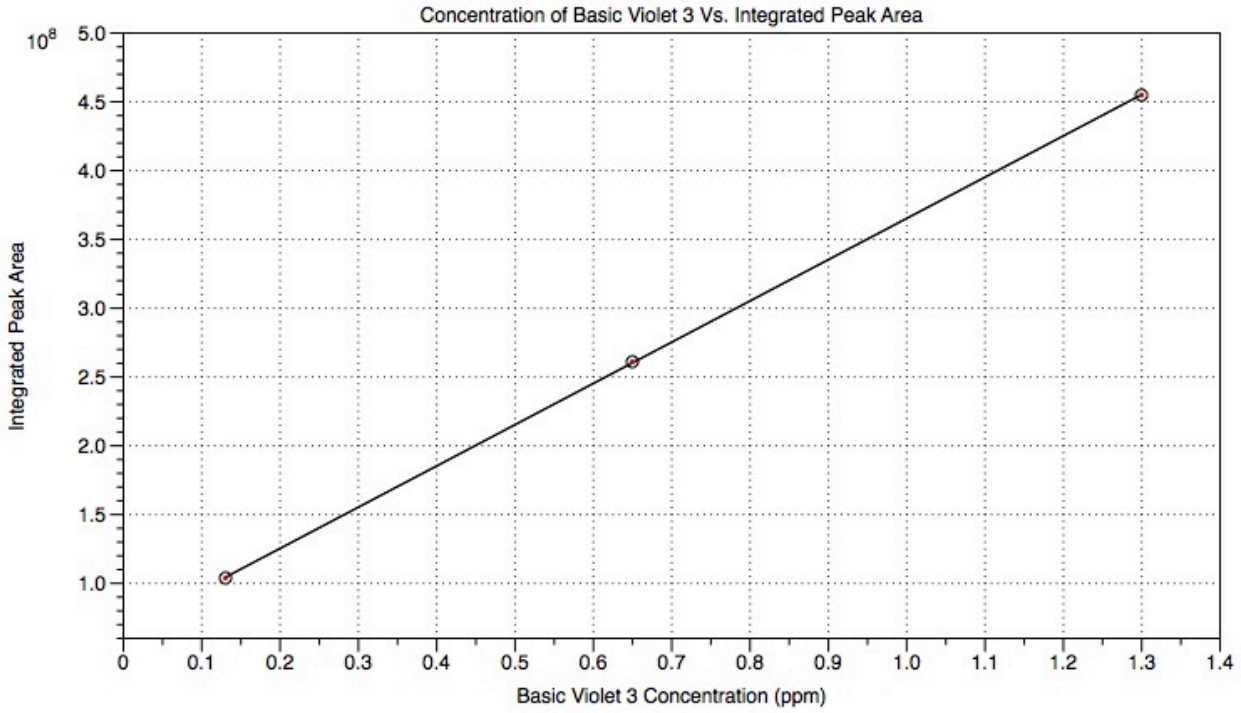

Figure 23: Concentration of Basic Violet 3 standard vs. integrated peak area

Method 2 (+0 V)

The additive used in Method 2 was 0.00041% reagent grade sodium acetate (0.45 µM) in filtered methanol. The optimized sodium acetate concentration allowed adduct formation by many of the ink vehicles and other additives, such as glycols and alcohols. When the concentration of sodium acetate used exceeded 0.00041%, a large increase in background noise was observed. Identification of vehicle ions in samples of gel inks was not successful in the positive mode (+120V with 4.1% glacial acetic acid) or in negative mode (-120 V with 4.1% triethanolamine). However, with a very dilute sodium acetate additive concentration, characteristic mass spectral patterns were observed in both ink samples and vehicle standards that were consistent with the sodium adducts. Sodium adducts tend to exhibit a characteristic peak pattern which includes peaks spaced m/z 44 units apart. Figure 24 shows the characteristic pattern for PEG 400 standard, when a minute concentration of sodium acetate is added as the post column additive.

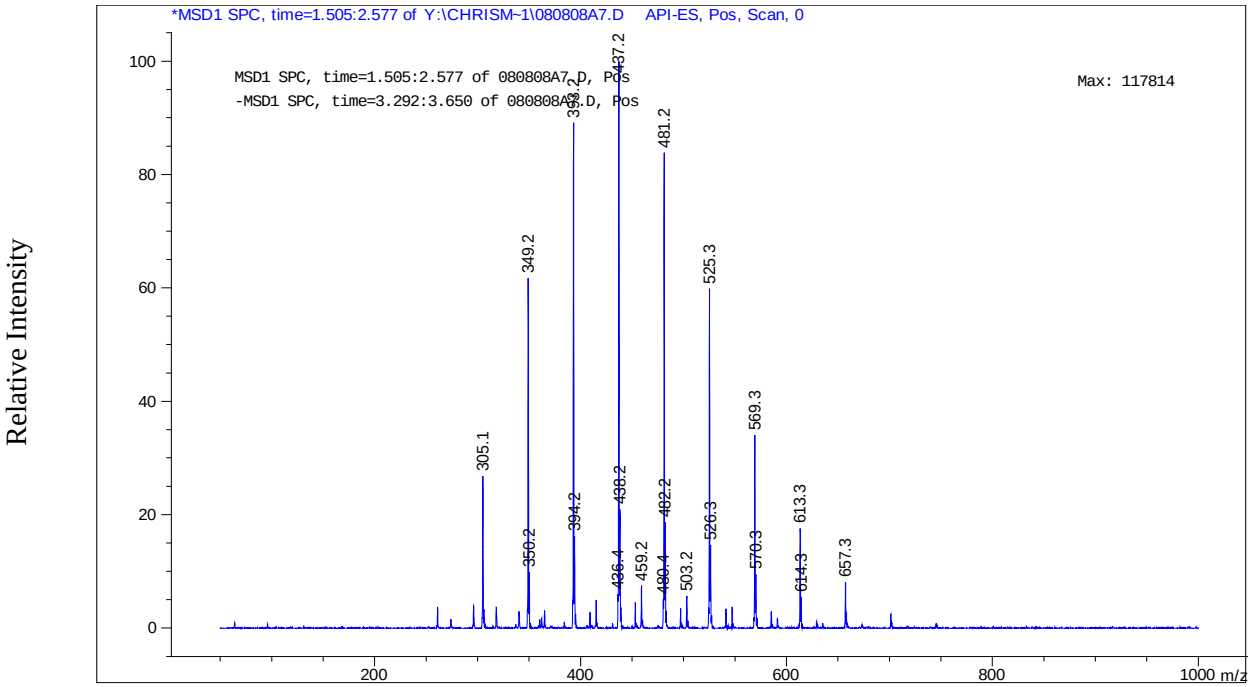

Figure 24: Polyethylene glycol (PEG 400) mass spectrum gathered using Method 2, illustrating sodium adduct presence the characteristic of spacing 44 m/z units apart [PEG-H+44n]

Method 3 (-120V)

Triethylamine (HPLC grade) was used at 4.1% concentration to ionize the Acid and solvent dye standards. Method 3 allowed several similar ballpoint inks to be distinguished from each other based only on Basic Violet and presence or absence of the 352 m/z peak. Deprotonated ions $[M-H]^-$ are typically observed with Method 3 with the use of a negative applied voltage. See Table 9 for some typically encountered ions with this method.

Volatile Persistence Study

The length of time after deposition that volatiles can be extracted from ink-on-paper samples and subsequently detected with direct infusion ESI-MS analysis has been studied. Samples consisted of an ink mark drawn on white paper with a pen from each of the three ink

categories. The analysis was performed using exclusively Method 2, as volatile/vehicle components can be observed as their sodium adducts when sodium acetate is added with zero applied fragmentor voltage. Each ink was sampled at the time of deposition, one hour, one day, and one week dry times. Results of direct infusion ESI-MS analysis at the deposition time and six hours after deposition are shown in Figure 27.

Analysis of Standards

Basic and Disperse Type Dye Analysis

Basic and disperse dye standards listed in Table 3 and 4 were examined by direct infusion ESI-MS analysis. Stock solutions of listed dyes were created in filtered methanol with concentrations ranging from 5.0 ppm to 6.5 ppm. These standard solutions were analyzed using (Method 1). The major ions are given in Table 7.

Acid and Solvent Type Dye Analysis

Standard solutions of commonly used acid and disperse type dyes were made in methanol and analyzed by direct infusion ESI-MS. Method 3 was used with these types of dyes as they responded best overall to these experimental conditions. Major ions observed can be seen in Table 9.

Vehicle Analysis

Additives collectively called the ink's vehicle were diluted with methanol and analyzed with direct infusion ESI-MS methods. These vehicle ions gave the greatest instrumental response with Method 2. Table 8 shows major ions observed from the vehicles and tables 7, 8 and 9 show some selected direct infusion ESI-MS spectra associated with these ions.

Table 7: Ions observed with Method 1 analysis

Standard Solution	Molecular Mass	CAS Number	Ion/fragment	m/z	Limit of Detection
Triethanolamine	149	102-71-6	[M+H]+	150	4.55
			[M+Na]+	172	
Glycerin	92	56-81-5	[M+Na]+	115	10
			[2M-2H20+Na]+	171	
Ethylene Glycol	62	107-21-1	[M+Na]+	85	5.67
2-Phenoxyethanol	138	122-99-6	[M+Na]+	161	8.41
Diethyl Phthalate	222	117-81-7	[M+Na]+	245	3.66
				327	
			[2M+Na]	467	
Benzotriazole	119	95-14-7	[M+Na]+	142	5.41

Table 8: Ions observed with Method 2 analysis

Dye Standard	Molecular Mass	CAS Number	Ion/Fragment	Ion m/z	LOD
Solvent Orange 3	212	495-54-5	[M+H]+	213	
			[M-NH2]	196	
			[M-(N-Ph)]+	121	
			[N=N-Ph]+	105	
Disperse Orange 25	323	31482-56-1	[M+H]+	324	
Basic Violet 1	393	8004-87-3	[M-Cl]+	358	3.0
			[M-Cl-CH3+H]+	344	
			[M-Cl-2(CH3)+H]+	330	
Basic Violet 3	372	548-62-9	[M-Cl]+	372	
Basic Red 1	478	989-38-8	[M-Cl]+	443	
			[M-Cl-CH3CH2+H]+	415	
Basic Violet 10	478	81-88-9	[M-Cl]+	443	
			[M-Cl-CH3+H]+	465	
			[M-Cl-CH3CH2+H]+	415	
Solvent Black 3	456	4197-25-5	[M+H]+	457	
			[M+Na]+	479	
			[M-(N=N-Ph)+2H]+	353	
Basic Blue 7	513	2390-60-5	[M-Cl]+	478	
			[M-Cl-CH3CH2+H]+	450	

Table 9: Ions observed with Method 3 analysis

Standard Solution	Molecular Mass	CAS Number	Ion/Fragment	Ion m/z	LOD
Acid Yellow 23	537	1943-21-0		134	
				198	
				219	
			[M-H]-	533	
Acid Yellow 73	376	518-47-8	[M-Na]-	353	
			[M-2Na+H]-	331	
				407	
				285	
Acid Blue 92	748	3861-73-2	[M-H]-	694	
			[M-Na]-	314	
			[M-2Na+H]-	650	
			[M-3Na+2H]-	628	
			[M-3Na+H]2-	314	
			[M-2Na]2-	324	
			[M-3Na+H]2- -O	298	
			SO3-	80	
Acid Yellow 3	477	8004-92-0	[M-Na]-	454	
			[M-SO3-Na]-	374	
			[M-2(SO3-Na)+H]-	352	
Acid Red 87	692	17372-42-1	[M-2Na+H]-	647	
			[M-2Na-Br+H]-	567	
Acid Yellow 36	374	587-98-4	[M+H]+	374	2.5
			[M-Na]-	352	
Acid Blue 9	792	3844-45-9	[M-H]-	769	
			[M-2Na+H]-	747	
			[M-2Na]2-	373	
			[NH-CH2-Ph-SO3]-	185	
			[CH2-Ph-SO3]	170	
			[Ph-SO3]-	157	
Acid Red 51	879	568-63-8	[M-2Na+H]-	835	
			[M-Na]-	857	
Solvent Orange 3	212	495-54-5	[M-H]-	211	
			[(N=N-Ph)-H]-	104	
Solvent Black 3	456	4197-25-5	[M-H]-	455	

Photochemical Experiments

Light Exposure Effects

Pen 1 was used to study the effect of exposure to ultraviolet light on pen-on-paper marks. Exposure to UV radiation is sometimes called “artificial aging,” or UV accelerated aging of an ink [22]. Pen 1 contains mainly Basic Violet 3 as its colorant, thus the effects of UV exposure on a system containing all the ink components could be observed. Exposure periods were twenty-four hours in length, samples were analyzed after each exposure period and analyzed using Method 1. Method 1 allowed changes in Basic Violet 3 to be observed and compared across all photolysis periods, Figure 25.

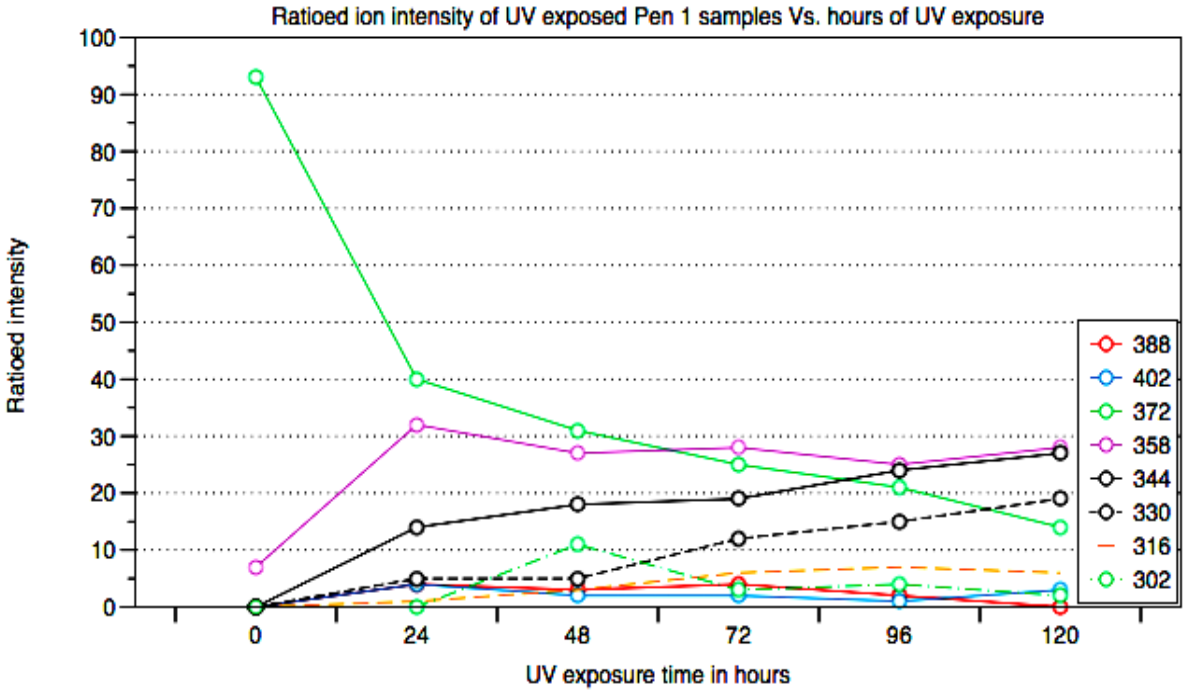

Figure 25: Change in relative intensities of ions produced during photolysis of pen 1 ink. Total ion intensity at each exposure time is normalized to 100%.

Figure 26 shows the mass spectrum of the dye from pen 1, when extracted at t=0, i.e., before light exposure. The spectrum contains primarily BV3 (m/z 372). Figure 27 shows the mass spectrum of the dye from pen 1 after five days of photolysis. The spectrum in figure 27 contains a series of peaks corresponding to the sequential loss of methyl groups from the BV3 molecules.

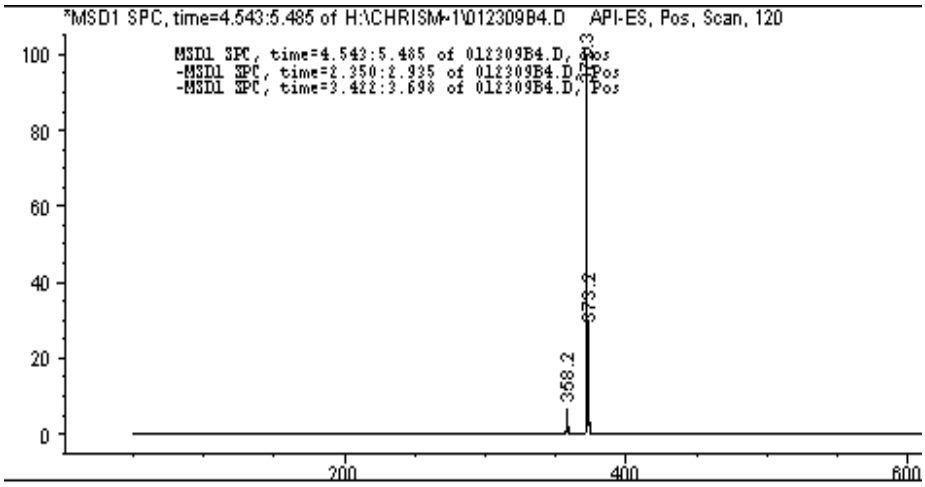

Figure 26: Mass spectrum showing time zero (no UV exposure) Pen 1. Photolysis sequence is BV3 (372 m/z) →CV (358 m/z) →MV (344 m/z) as methyl groups (15 m/z) are removed and replaced by hydrogen

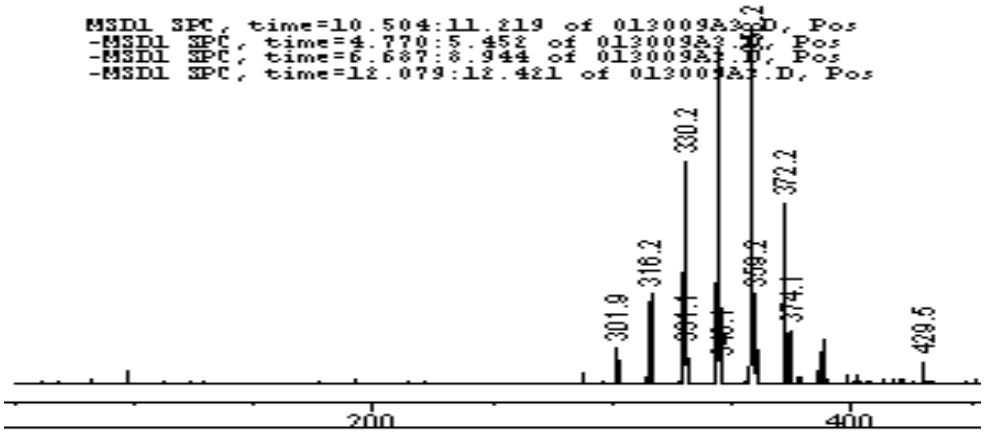

Figure 27: Mass spectrum showing Pen 1 after 5 days (120 Hours of UV exposure)

Potassium Ferrioxalate Actinometry

An actinometer solution was also exposed in the same photoreactor as the Pen 1 samples. After exposure, the potassium ferrioxalate solution was complexed with 1,10-phenanthroline and the UV absorbance of the complex could be determined. The UV absorbance data collected allowed the photon flux to be calculated, see Figure 28. The spectral output of the RPR and other lamps used in this procedure can be seen in Figures 16. The calculated photon flux of the RPR photoreactor is $5.7x10^{14}$ photons/sec*cm^{-2} impinging on the ink samples used in this study.

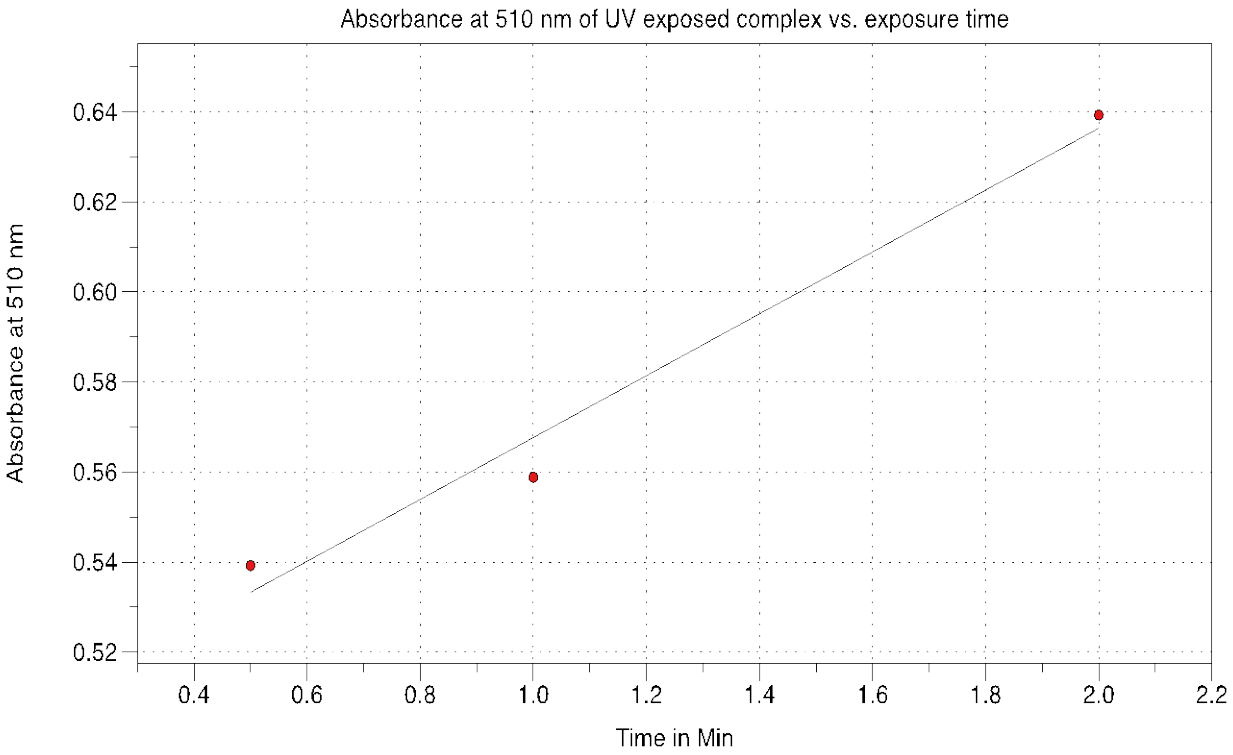

Figure 28: Actinometer exposure time vs. absorbance at 510nm of 1, 10-phenanthroline complex

Method Validation

Triplicate Analysis of Reference Pen Collection

The collection of pens was analyzed in triplicate and compared to previously gathered direct infusion ESI-MS data (Williams et al., Pens 1-12 and 17-22). Eleven more inks were added to the reference collection and analyzed in triplicate with all three analytical methods. Table 7, 8, and 9 shows major ions found from the previous research and the eleven pens added to the collection.

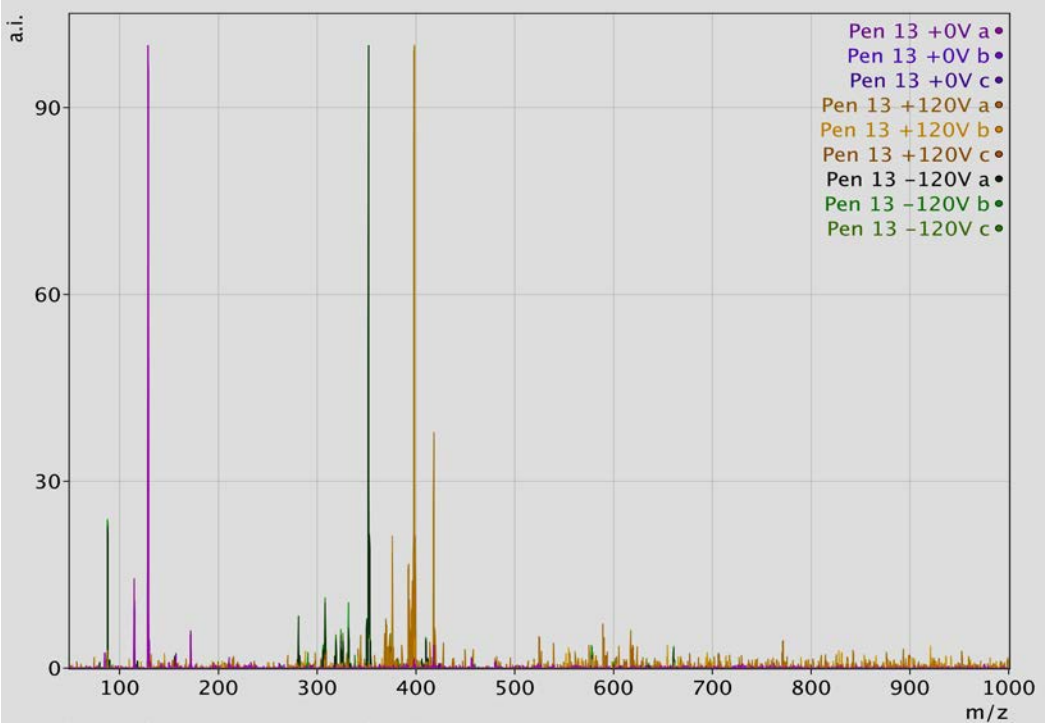

Figure 29: Fingerprint spectrum of Pen 13, with combined Methods 1, 2, and 3

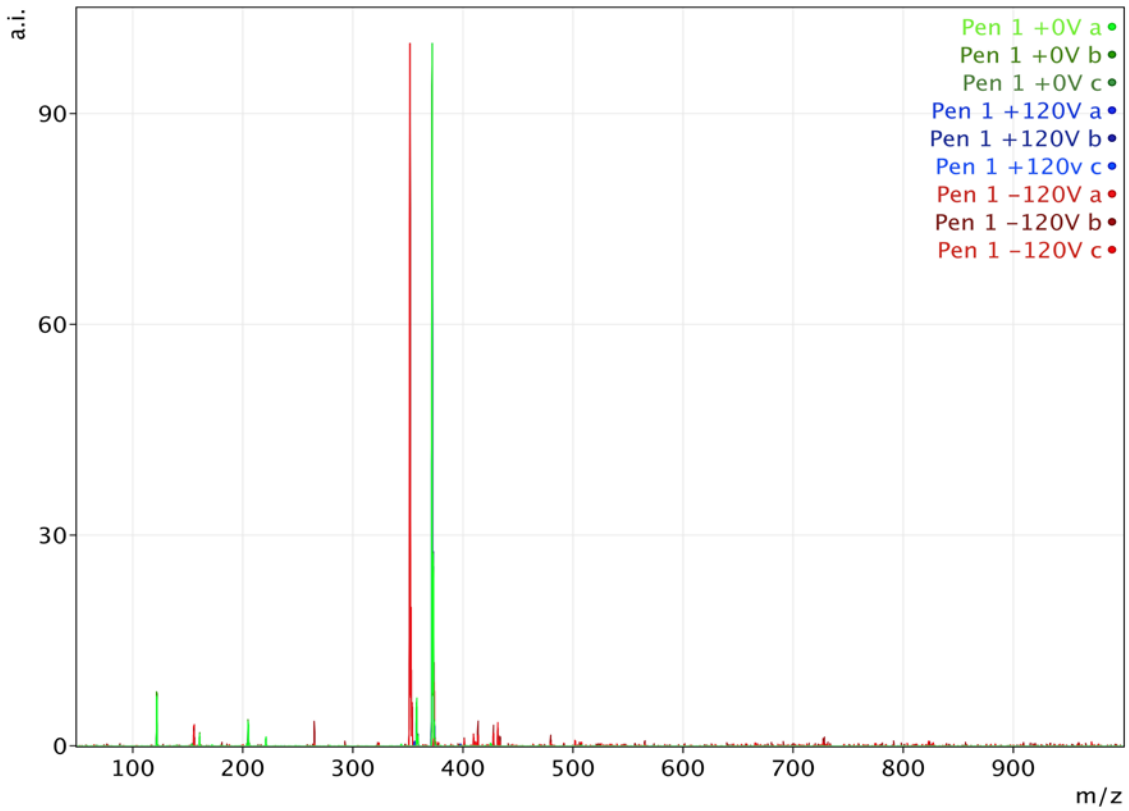

Figure 30: Fingerprint spectrum of Pen 1, with combined Methods 1, 2, and 3

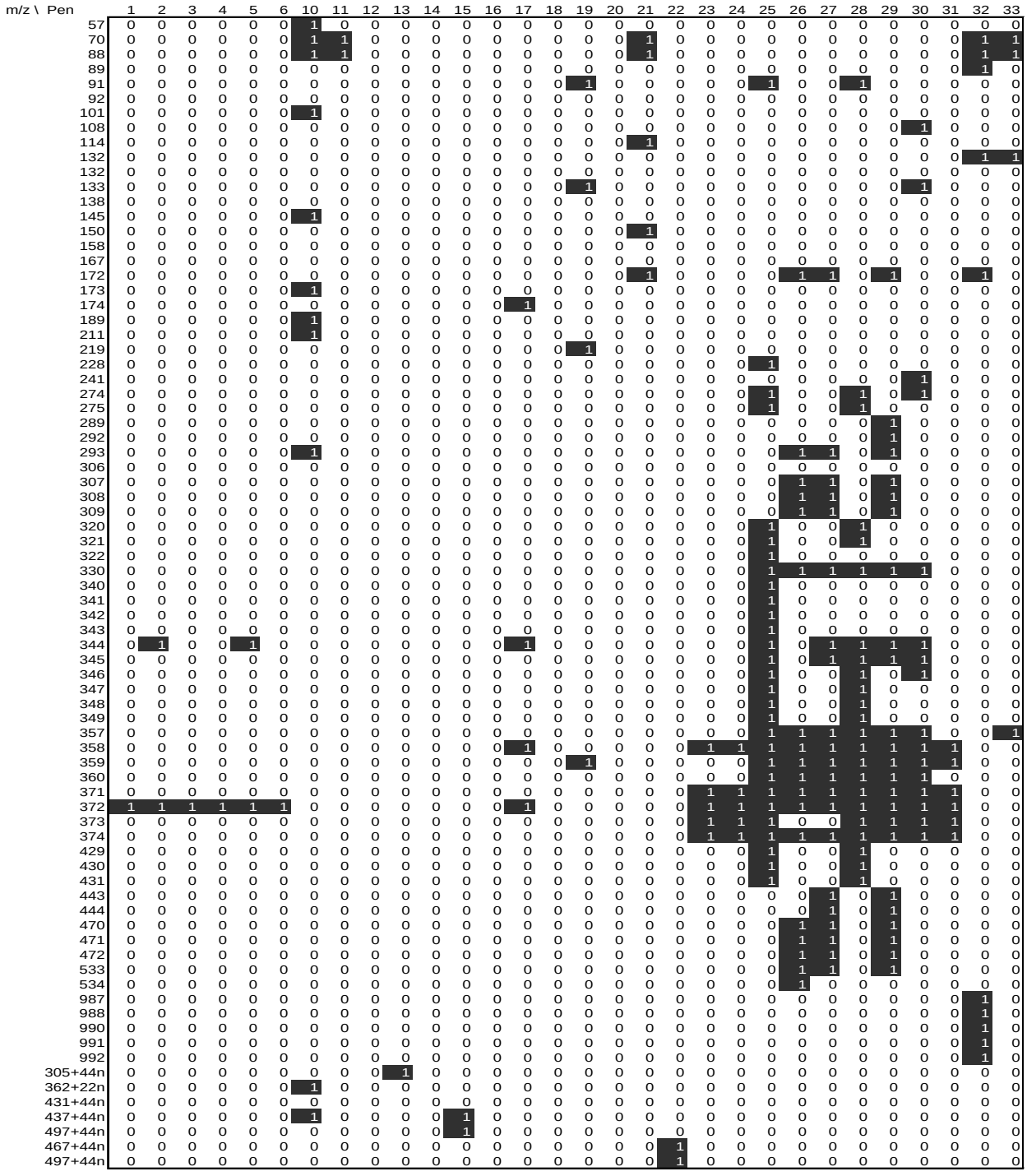

Figure 31: Ions observed with Method 1 for triplicate analysis

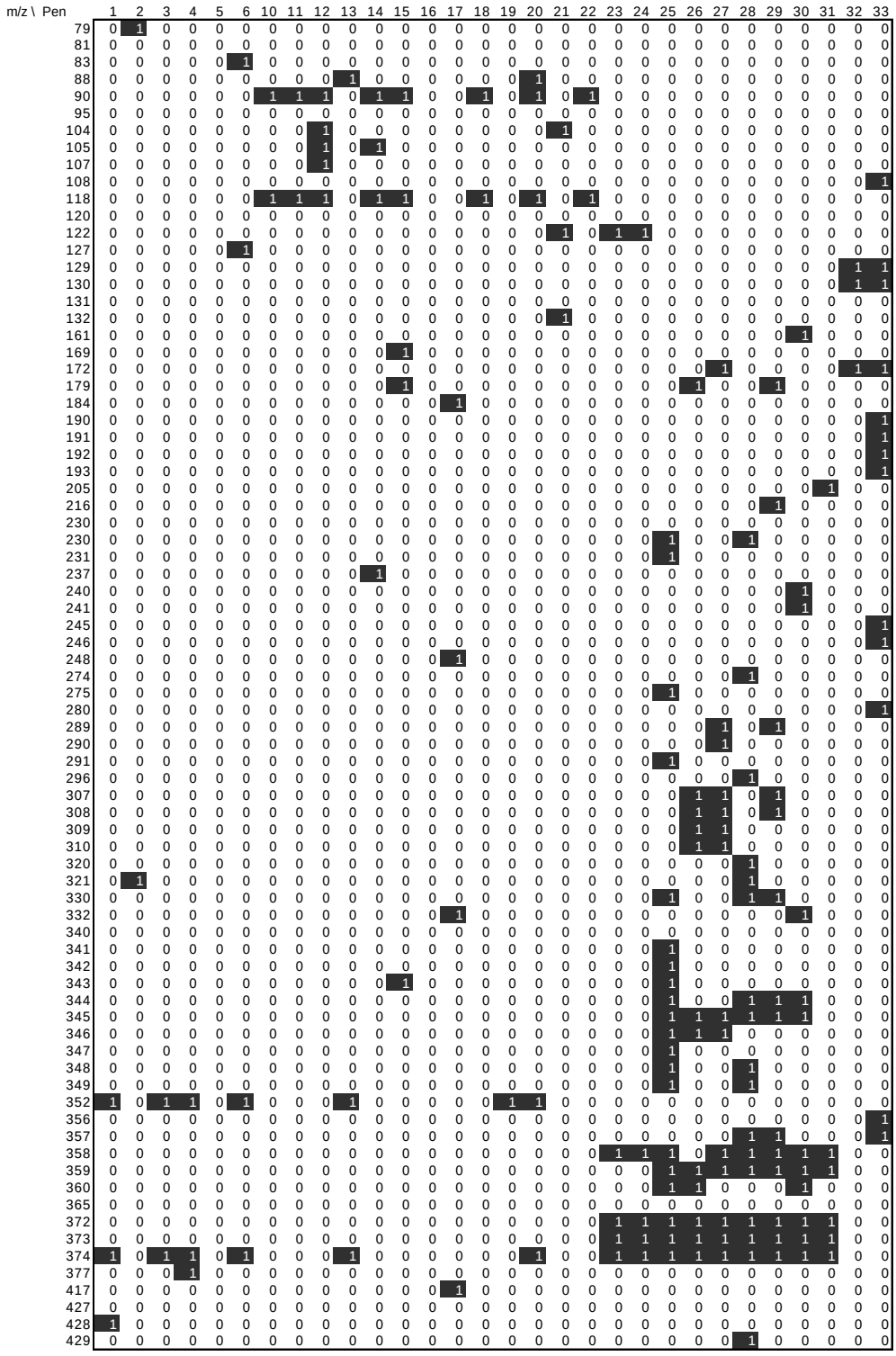

Figure 32: Ions observed with Method 2 for triplicate analysis

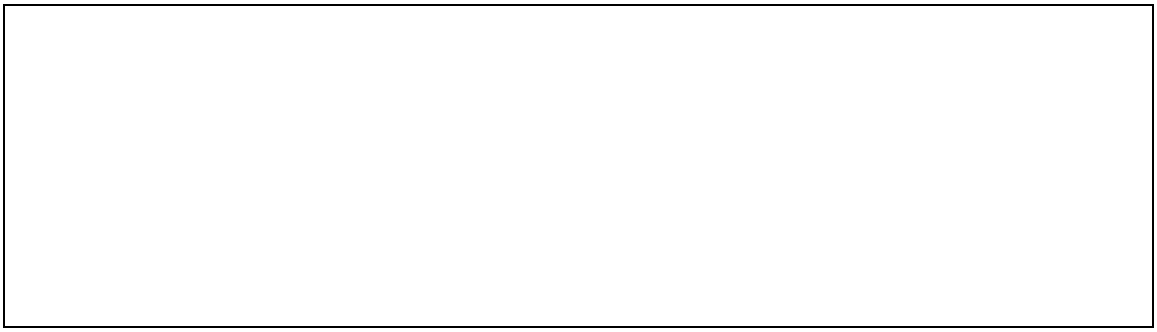

Figure 32: Ions observed with Method 2 for triplicate analysis (continued)

m/z \ Pen	1	2	3	4	5	6	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
85	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
90	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
108	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0
115	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0
117	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
120	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
122	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
127	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
129	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
133	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
140	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
142	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
145	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
150	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
154	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
156	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0
157	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
161	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
162	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	1	0	0	1
163	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	1	0	0	1
164	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	1	0	0	0
165	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	1	0	0	0
172	0	0	0	0	0	0	1	1	1	1	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
173	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
174	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
193	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
196	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
205	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
213	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
216	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
219	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
229	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
230	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
245	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
266	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
266	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1
274	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
296	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
326	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
330	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
340	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
342	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
344	0	1	1	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
352	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	1	0	0
353	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	1	0	0
354	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	1	0	0
358	1	1	1	1	1	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Figure 33: Ions observed with Method 3 for triplicate analysis

m/z \ Pen	1	2	3	4	5	6	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
359	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
372	1	1	1	1	1	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
398	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
415	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0
418	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
427	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0
429	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
446	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1
448	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1
468	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1
468	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
469	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1
470	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1
512	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
513	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
514	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
586	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
374	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	1	0	0
296+22n	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
305+44n	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
326+22n	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
356+22n	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
415+44n	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
431+44n	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
437+44n	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
467+44n	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
541+44n	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
326+22n	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
403+44n	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
467+44n	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
474+22n	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
488+22n	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
497+44n	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
607+44n	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0

Figure 33: Ions observed with Method 3 for triplicate analysis (continued)

	1	2	3	4	5	6	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
1																														
2	1																													
3	1	0																												
4	1	0	1																											
5	1	1	1	1																										
6	1	0	1	1	0																									
10	0	0	0	0	0	0																								
11	0	0	0	0	0	0	0																							
12	0	0	0	0	0	0	0	0																						
13	0	0	0	0	0	0	0	0	1																					
14	0	0	0	0	0	0	0	0	1	0																				
15	0	0	0	0	0	0	0	0	1	0	1																			
16	0	0	0	0	0	0	0	0	1	0	1	0																		
17	1	1	1	1	1	1	0	0	1	0	1	0	1																	
18	0	0	0	0	0	0	0	0	1	0	1	0	1	0																
19	0	0	0	0	0	0	0	0	1	0	1	0	1	0	1															
20	0	0	0	0	0	0	0	0	1	0	1	0	1	0	1	0														
21	0	0	0	0	0	0	0	1	1	0	1	0	1	0	1	0	1													
22	0	0	0	0	0	0	0	0	1	0	1	0	1	0	1	0	1	0												
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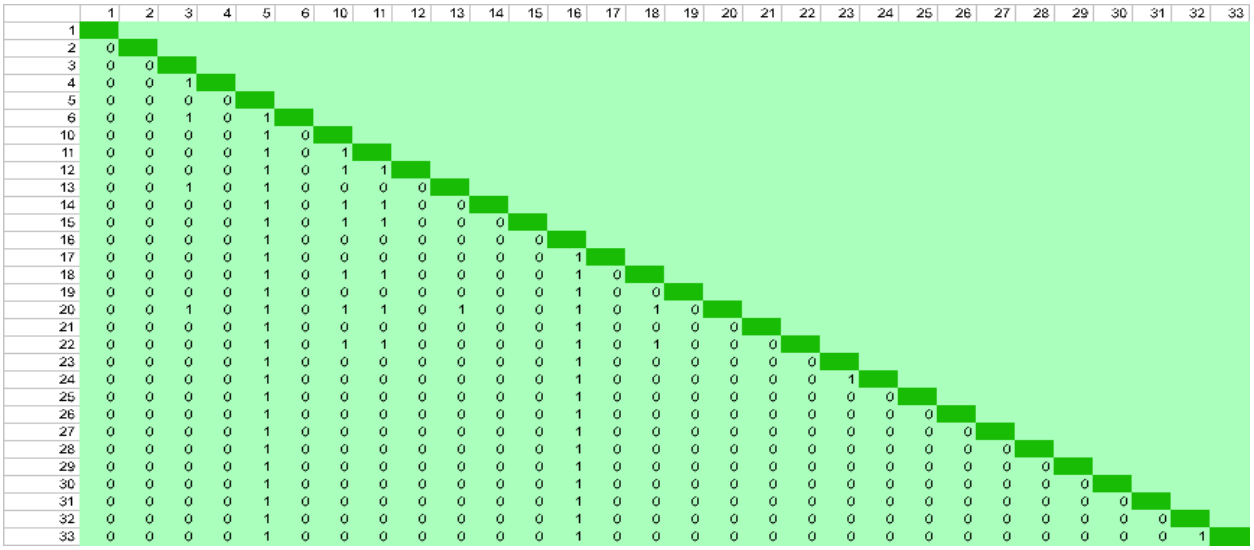
25	1	1	1	1	1	1	0	0	1	0	1	0	1	0	1	0	1	0	0	1	1									
26	1	0	1	1	0	1	0	0	1	0	1	0	1	0	1	0	1	0	0	0	0	0								
27	1	1	1	1	1	1	0	0	1	0	1	0	1	0	1	0	1	0	0	0	0	0	0							
28	1	1	1	1	1	1	0	0	1	0	1	0	1	0	1	0	1	0	0	1	1	0	0	0						
29	1	1	1	1	1	1	0	0	1	0	1	0	1	0	1	0	1	0	0	1	1	0	0	1	0					
30	1	1	1	1	1	1	0	0	1	0	1	0	1	0	1	0	1	0	0	1	1	0	0	0	0	0				
31	1	0	1	1	0	1	0	0	1	0	1	0	1	0	1	0	1	0	0	1	1	0	0	0	0	0	0			
32	0	0	0	0	0	0	0	1	1	0	1	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0		
33	0	0	0	0	0	0	0	1	1	0	1	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	

Figure 34: Pair-wise comparisons of Method 1 results (1 = not discriminated Method 1)



	1	2	3	4	5	6	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
1																														
2	0																													
3	0	0																												
4	0	0	1																											
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18	0	0	0	0	1	0	1	1	0	0	0	0	1	0																
19	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0															
20	0	0	1	0	1	0	1	1	0	1	0	0	1	0	1	0														
21	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0													
22	0	0	0	0	1	0	1	1	0	0	0	0	1	0	1	0	0	0												
23	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0											
24	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1										
25	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0									
26	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0								
27	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0							
28	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0						
29	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0					
30	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0				
31	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0			
32	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
33	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

Figure 35: Pair-wise comparisons of Method 2 results

Out of 435 pair-wise comparisons 429 pairs could be discriminated from each other using the combined three methods, seen in figures 34 and 35. This is a 98.6% discrimination with the combined analysis scheme. A number 1 in Figures 34 & 35 means the ions observed using the described methods indicates an area of non-discrimination with the single method. However, when the observed ion data from the other two methods are combined, the overall discriminating power of the direct infusion ESI-MS methods are realized. To visualize the ion data gathered from the three methods, a fingerprint spectrum is generated from the Method 1, 2, and 3 ions observed the ink sample's mass spectrum. Examples of two fingerprint spectra can be seen in Figures 29 and 30, which use the three combined mass spectra of Pen 1 and Pen 13 ink samples.

Multipack Analysis

Multipacks of pens with various colors close to black were analyzed by the direct infusion ESI-MS methods. Only gel and ballpoint inks were used, as rollerball inks tend to have more compositional variation and were excluded from this portion of the study. The list of pens

used for this portion and colors can be seen in Table 6. Several of these inks' mass spectra were compared to similar black inks' mass spectra from the reference collection Figure 36.

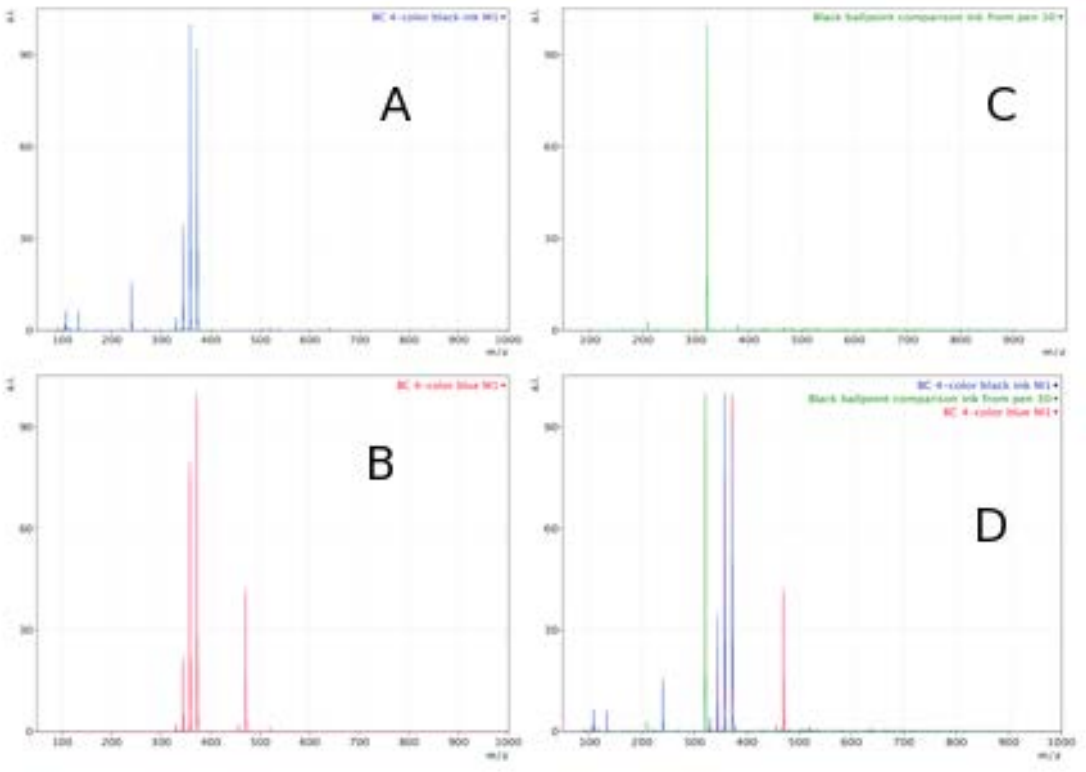


Figure 36: Comparison of Method 1 results for BC 4-Color Black (A), BC 4-Color Blue (B), Pen 3 (C) and a complied comparison spectrum (D)

Intersecting and Overlapping Ink Strokes

Overlapping strokes made with Pens 3 and 5 were examined with the combined three direct infusion ESI-MS methods. The "altered" document was sampled as detailed in Figure 22. Samples were collected from portions that were known to have only Pen 3 ink, known to only have Pen 5 ink, or known to be a portion of overlap of the two inks.

Pen 5 Only Sample (Figure 37)

The Pen 5 only sample's mass spectrum with Method 1 and 2 consists only of 372 m/z and 358 m/z ions in 100% and 11% relative abundances, respectively. While the other analysis mode, Method 3, shows 373 m/z and 352 m/z ions in 7% and 100% relative abundance levels.

Pen 3 Only Samples (Figure 38)

The Pen 3 ink sample's mass spectrum contained also 372 m/z and 358 m/z ions with 76% and 100% relative abundance values, which is in contrast to the 100:11 ratio seen in Pen 5 Method 1 mass spectra.

The Method 1 spectrum also exhibits 174, 340, 342, 344, and 345 m/z ions, which were not observed in Pen 5. The Method 2 spectrum of Pen 3 contained 274, 296, 330, 340, and 344 m/z peaks. Method 3 analysis of Pen 3 only sample showed a single peak at 713 m/z with 100% relative intensity.

Overlapped Areas

Sample 1

Overlap area sample 1 exhibits a spectrum similar to Pen 3 only samples. Sample 1's collected spectra have the same ions as Pen 3 and in very similar relative abundance values.

Sample 2

Sample 2, however, is more similar to the Pen 5 only spectra. With Method 1, sample 2, exhibits a relative abundance of 100% for the 372 m/z ion peak, and a significantly smaller 358 m/z ion peak when analyzed with Method 1 conditions. Sample 2 also exhibits a 713 m/z peak with Method 3, which is seen in the Pen 3 only samples. The Method 3 analysis of sample 2,

does not elicit a 352 m/z peak similar to Pen 5. Method 3 does show a 100% relative intensity peak for 274 m/z ion, similar to Pen 5 only sample's Method 3 mass spectrum. Method 1 results for sample 2 also contain 344 m/z and 147 m/z peaks, similar to Pen 3's Method 1 results.

Method	m/z	Relative abundance
+120 V	174.1	19.155
	340.1	7.845
	342.1	3.429
	344.2	38.81
	345.3	6.172
	358.2	100
	372.2	76.012
+0V	274.2	8.804
	296.2	6.567
	330	3.49
	340.1	23.414
	342.1	7.343
	344.2	48.573
	358.2	100
	372.2	89.98
-120V	713	100

Note: Max counts for Method 3 are three orders of magnitude lower than for Method 1 or Method 2

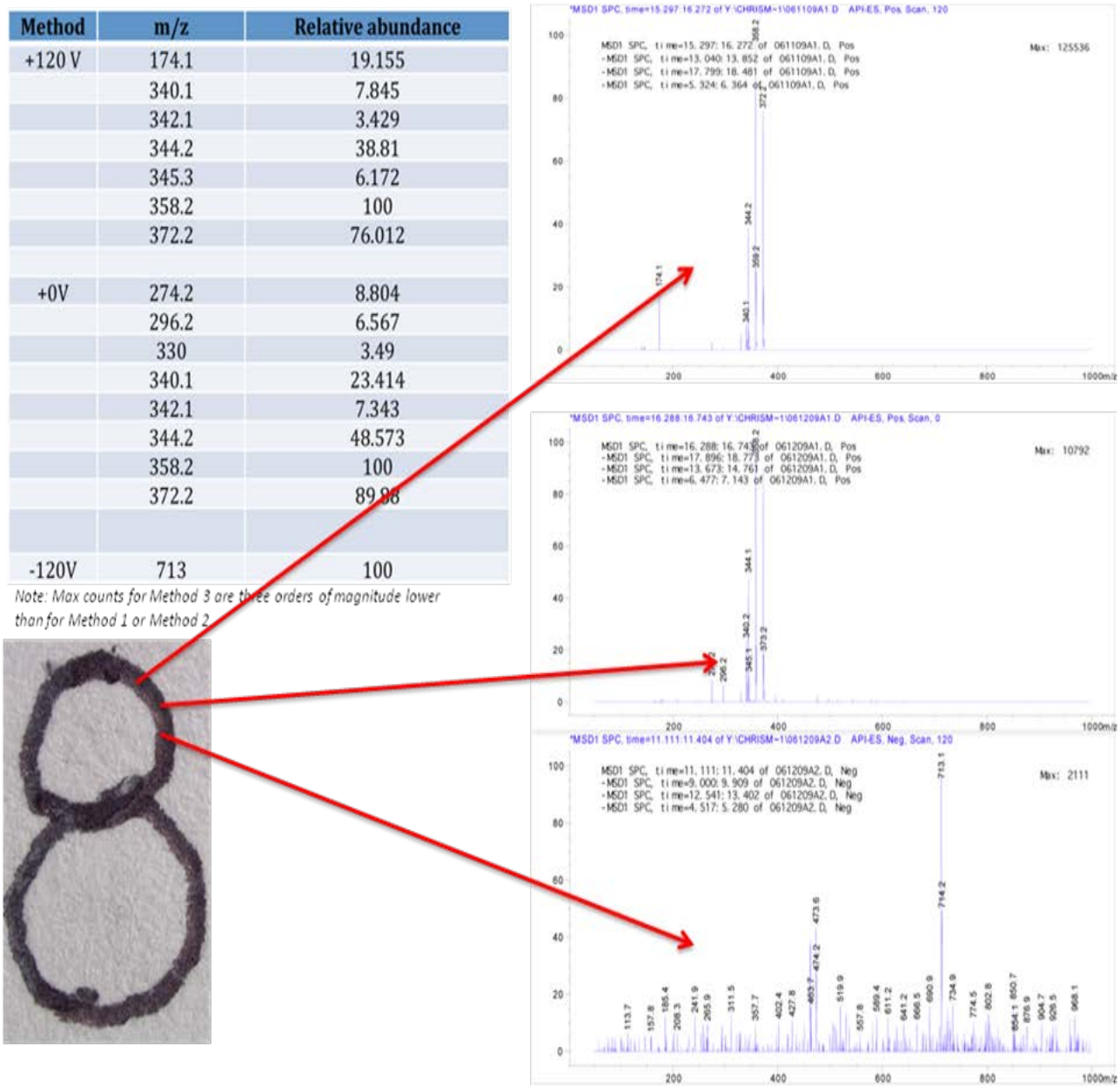
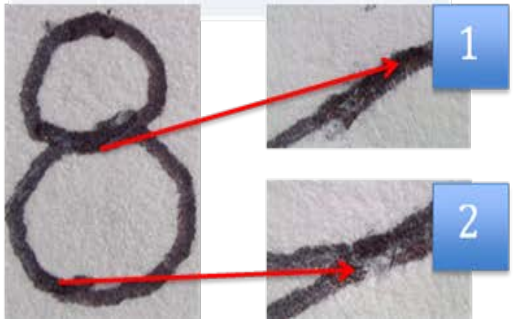


Figure 37: Results, sampling locations, mass spectra, and observed ions pen 3 only section of altered document

mode	Pen	ion	Relative abundance	Relative abundance
+120V	5	147.1	20.86	4.069
		340.1	5.981	1.5514
		342.2	2.607	0.6925
		344.2	39.128	9.549
		345.4	2.829	1.27
		358.2	100	33.002
		372.2	68.466	100
+0V	5	274.2	9.252	100
		296.2	3.834	7.042
		330	4.386	5.58
		340.1	20.648	
		342.1	6.664	2.528
		344.2	44.027	2.792
		358.2	100	1.194
		372.2	80.24	8.467
				1.686
-120V	5	713>60		14.248



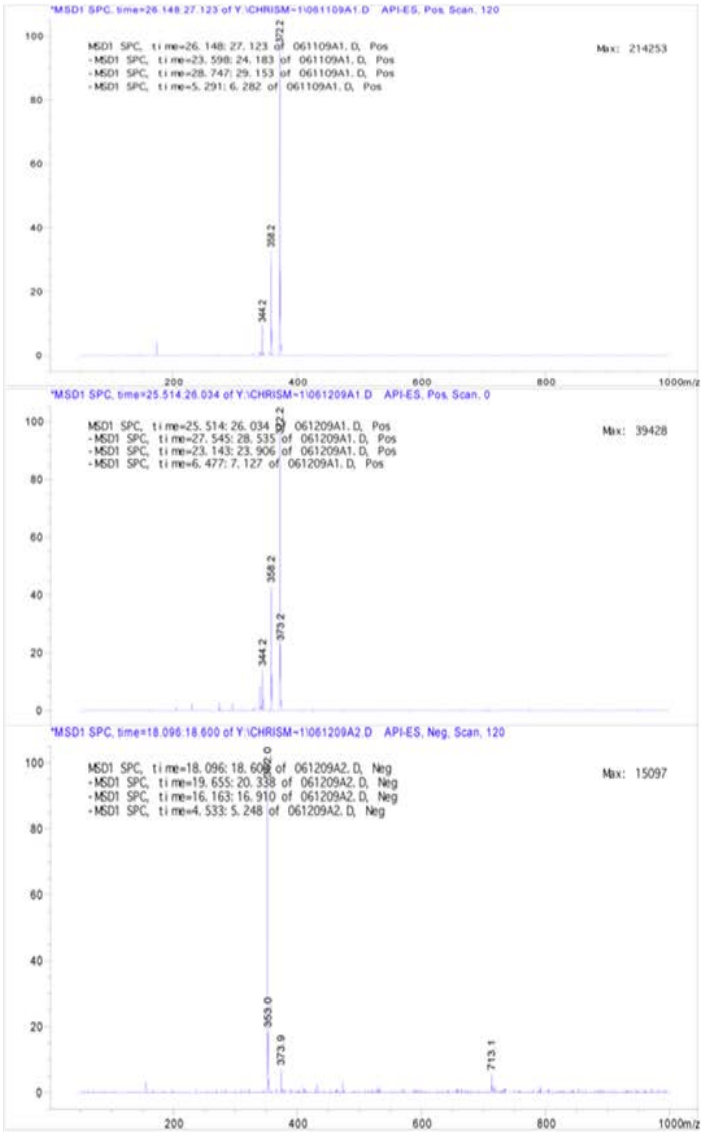
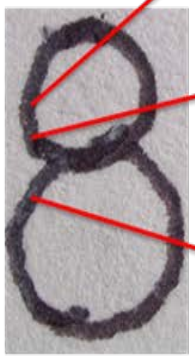


Figure 38: Results, sampling locations, mass spectra, and observed ions overlap sections 1 and 2

mode	Pen	Sample location	ion	Relative abundance
+120V	3	3	358.2	11.011
			372	100
+0V	3	3	358.2	7.257
			372.2	100
-120V	3	3	352	100
			373.9	7.62



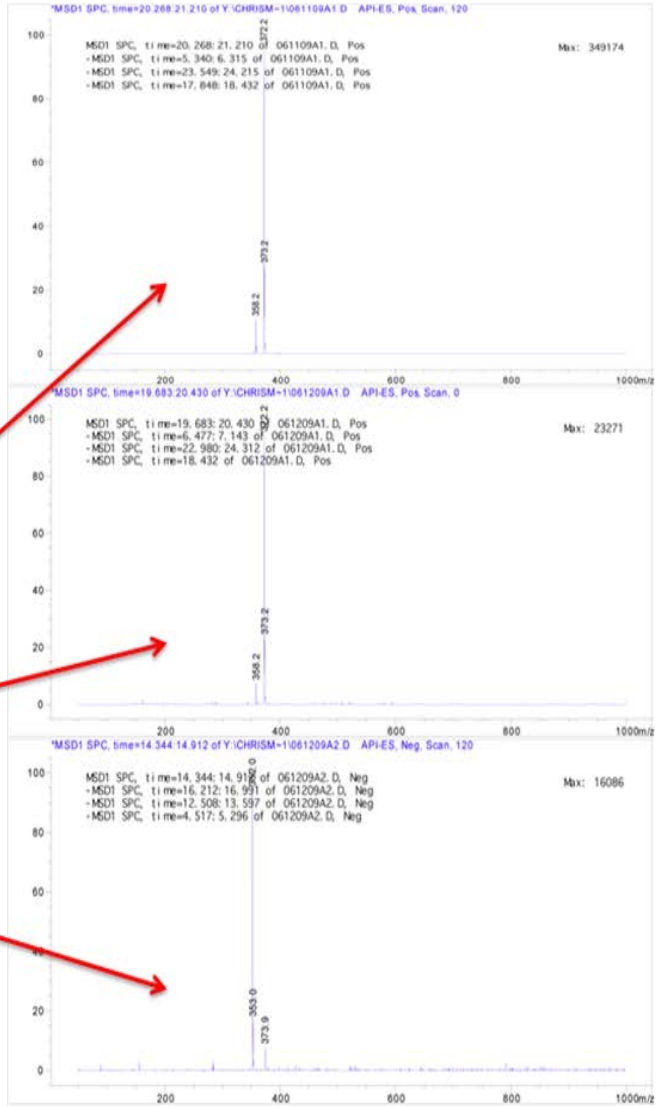


Figure 39: Results, sampling locations, mass spectra, and observed ions of Pen 5

CHAPTER FOUR: DISCUSSION

Direct Infusion ESI-MS Analysis

The use of the three combined direct infusion ESI-MS methods allowed differentiation of 99% of the 435 unique pairwise comparisons of the 30 inks examined in this study. The best way to differentiate between ink samples is to combine the spectra from each method and compare the combined spectra from different samples. Additionally, each method has been tuned to provide the best operating parameters for dye and vehicle detection. Each method has a specific subset of target molecules, which respond in a similar manner to the instrumental conditions described.

It is clear, based on the results presented in this thesis, that ESI-MS direct infusion analysis methods and associated extraction procedures used with Method 1, Method 2, and Method 3 are able to differentiate a majority of fresh ink samples. The effects of light exposure, solvent drying, temperature, and issues related to ink composition and storage conditions of the samples in question can influence sample discrimination.

Volatile Persistence Study

Previous research reported the detection of ink volatiles for an extended period of time after deposition, and the ability to determine this time interval [21]. Using the direct infusion ESI-MS method, some of the volatiles were not present in detectable amounts in tested samples; others were only detectable for less than one week. The previous research analyzed for volatiles like 2-phenoxyethanol, which are found in many commonly used household products such as lotion. Using the direct infusion ESI-MS methods described in this work, a majority of volatiles are not detected after drying periods of a week or less. The importance of volatile identification

for gel inks is that there are few analytical instrumental methods which can identify pigments. Pigments in ink bind to the paper fiber surface and are not meant to be removed; however, the spectra of the colored gel and some black rollerball inks in this study indicate that there are identifiable mass spectral peaks that allow some differentiation of inks that only differ in pigment color.

Analysis of Standards

The instrumental response of some Basic dyes in the positive mode was relatively large, even without a pre-ionization chamber additive. Typically these dyes are in charged states (i.e. Hexamethylpararosanaline hydrochloride salt or Basic Violet 3 added to solvents like water or polyethylene glycol). ESI-MS methods are well suited for detecting and differentiating between molecules that contain a mixture of charge states, as some dyes are known to be used in a more than one charge state. While laser desorption-mass spectrometry (LD-MS) is one way to detect pigment molecules, detection of molecules with a charge of more than one, the instruments are expensive and not readily available and desorption matrix is required.

Dyes that did not respond using only mobile phase for analysis, showed a general trend of increased instrumental response when a mobile phase additive was used. The disperse and solvent type dyes typically did not exhibit a response with the intensity of the basic and acid dye classes. The addition of acetic acid (Method 1) or triethylamine (Method 2) allowed many of the lower resonding dyes to be observed with direct infusion ESI-MS methods.

Vehicle Analysis

Vehicle components are volatile and are intended to carry the dye or pigment onto the writing surface and then aid in binding to the document. Binders typically found in inks include

povidone (polyvinyl pyrrolidone), sucrose, and glycerol. The compounds used as binders are used in other applications as starting materials for polymerization reactions. Manufacturers know the average drying time of these glycol-based inks and how to manipulate the content and concentrations of these classes of solvents to achieve market favored products. The concentration of volatiles presented for analysis can fluctuate based on the time since deposition and storage conditions. The time since deposition could not be determined by using just the ion ratios of the hexamethylpararosanaline ion and its degradation products in an ink mass spectrum as the m/z 372 series see Figure 26. Determining the time since deposition by this method would require a knowledge of the document exposure to different light sources.

Photochemical Experiments

Light Exposure Effects

Analysis of Pen 1 with Method 1, which contains almost exclusively Basic Violet 3 as a colorant, was exposed to UV radiation in a Rayonette RPR 350 photoreactor for up to five days (120). The ink sample initially (t=0) contained in its mass spectrum after subtraction, m/z 372 and 358 ions with Method 1 instrumental conditions. As UV exposure continues to five days, the m/z 372 relative intensity dropped to 50%, 344 and 358 increased to approximately 100%. After one day of exposure in the photoreactor, ink from Pen 1 began to exhibit a 388 peak. The m/z 388 peak was not observed in any of the tin foil covered control samples. Figure 27 shows the shifting pattern of degradation products formed by UV exposure. Relative intensity values from ink samples exposed in the UV photoreactor for periods ranging from zero to five days show the gradual conversion of Basic Violet 3 to leucocrystal violet, which is more yellow in color and does not contain methyl groups attached to the three amino groups.

The method of differentiating and aging black ballpoint ink samples by the ratio of methylated vs. non-methylated amino groups on Basic Violet 3 proved to be an unreliable method. Figures 1 and 2 (chapter 1) show the results for ink that was exposed to a common desk lamp with an eco-friendly GE Helical light bulb. The ink was almost fully photo-bleached within 72 hours of exposure. The ratio of methylated to non-methylated amino groups in randomly selected "pure" BV 3 sample is variable. Additionally, use of the ratio of BV3 and degradation products for time since deposition determination does not take in to account the effects of the vehicle and the paper itself or other compound on the stability of the 372 m/z series.

Grimm, Siegel, and Allison used UV (λ of 254nm) accelerated artificial aging to assess dye degradation with LD-MS. A BIC black ballpoint pen was used to compare 6.25 hours of UV exposure with a 38-month controlled, naturally aged ink sample. The LD-MS experiments indicate the dye degradation of 6.25 hours shows a similar mass spectrum to the 36-month non-UV sample [6]. The UV artificial exposure experiments showed the degradation of BV3 to BV1. Aged sample types showed a similar narrowing of BV3, z/m 372 peak and an almost 50% increase in relative intensity of the 358 m/z peak corresponding to MV. In addition, both aged samples exhibited a 25-35% relative intensity 344 m/z peak and a roughly 3-9% relative intensity peak at 330 m/z from the replacement of three methyl groups by hydrogen seen with LC-MS methods. The control sample only showed the typical BV3 mass spectrum.

This degradation pattern seen by Grim, was also observed in ballpoint pens after only 24 hours of UV exposure, in this study. However the samples examined with no UV exposure did not exhibit a change in dye composition. The unexposed samples were on the same sheet of paper as the UV exposed samples, except they were covered tightly with aluminum foil. The degradation was caused by the UV exposure, not the heat generated by the photoreactor. The

unexposed Pen 1 samples exhibited no change in mass spectrum with sample treatment from 24 to 96 hours. This is observation agrees with Grim's claim that a ink samples stored in darkness exhibit a considerably less degradation of dyes and a different mass spectrum than those exposed to a light source. Unknown variables, including, variable storage conditions and ink-solvent, ink-paper, ink-ink interactions make an age determination from the mass spectral data, next to impossible. In this study, the exposure times were controlled and mass spectra were compared after each exposure period. With casework samples, the analyst has little expectation of knowing the time of light exposure, time of storage in darkness, or the photon flux of any incident light that has interacted or not interacted with a sample, especially if the documents are more than a few months old or older. This study demonstrates that a ballpoint ink on paper sample could be exposed to UV light in order to create writings that appear aged longer than the actual time since deposition of the ink on the paper.

The RPR photoreactor used in this study generates a narrow emission centered on 350 nm (figure 16). This photon flux is enough to photo-fade the samples rapidly. However the action of the desk lamp, which has a lower photon flux and broader spectral output, approximates the photo-fading action of sun light. The sun is spectral output reaching the surface depends on the location of the observer, season, cloud cover, and effects of atmospheric scattering and refracting. The desk lamp produced the yellowish color of leucocrystal violet in exposed samples within 72 hours.

Triplicate Analysis of Reference Pen Collection

Direct infusion ESI-MS analysis of inks used by Williams et al. (a subset of all inks used in this research) were in complete agreement with the mass spectral data for Pens 1-6, and 10-16

obtained in this work [2]. The triplicate analysis allows statistical treatment of collected data for comparisons.

Other Pen Analysis

As seen in Figures 36, 37 and 38, when samples contain areas where two different pens (i.e. Pen 3 and Pen 5) were used, it is possible to differentiate between the two pens based on the direct infusion ESI-MS methodology.

Gas chromatographic methods are not compatible with non-volatile ink components, however, liquid chromatography based methods allows the identification of dyes, salts, solvents, and some adduct molecules. A mass spectral fingerprint can be obtained using the three direct infusion ESI-MS methods described in the previous chapter.

Ideally, the forensic scientist would like to be able to identify a sample ink by comparing an extract mass spectral fingerprint to determine origin of the sample. Data collected using each method should be considered separate but linked information, as all three methods add data that can help to differentiate similar inks. Comparison of extracts from different samples is one goal of the quest for a standardized ink analysis method. This would allow the forensic scientist to assess the possibility that the two inks share a common origin. This determination can be affected by degradation and time since deposition.

In order to study the effect of time since deposition, a sample ink line was made on plain white copy paper and sampled at time intervals of zero minutes, thirty minutes, one hour, twenty-four hours, and one week after deposition. The three ink samples chosen to study the volatile component retention behavior of ink-on-paper samples included members from the three common ink classes. All inks contain some type of solvent to allow the colorant to be transferred

from the ink reservoir to the paper. Some solvents used in inks include PEG, benzyl alcohol, and ethanol. Past research indicated that GC-MS methods were capable of detecting ink volatiles after a period of months using extraction differences in strong and weak solvents. However, in the volatile persistence study reported here, a majority, of identified ink components were observed for less than a week when analyzed with direct infusion ESI-MS methods. The complexities of ink, its drying, decomposition, and interactions with paper make analysis after 48 hours difficult to interpret.

Some inks in the sample population were indistinguishable by direct infusion ESI-MS methods. An overall discrimination of 99% of the 435 possible pairwise comparisons among 30 pens was achieved. There are several possible explanations for the failed discriminations, the inks tested were actually identical inks, the inks were similar but undistinguishable (different batches or lots) or they are different inks.

Differentiation of inks is possible when they are compared on a similar time frame. Ink inside of a pen for the most part is not exposed to the environment until it is written on paper. Ink from the same pen, one sample deposited and extracted within 20 minutes then compared to a sample deposited 20 years earlier, would look quite different from each other with the direct infusion ESI-MS methods described. The environmental effects and the massive sample pool of inks need to be investigated before individualization of aged inks becomes a viable forensic practice. It was once an accepted or commonly held opinion that the extent of an ink's time since deposition could be determined by the presence or absence of a particular volatile molecule by GC-MS analysis [13, 14].

Determination of the extent of aging with ballpoint inks has also been attempted with LC-MS techniques like laser desorption ionization mass spectrometry (LDI-MS), MALDI, and

others using the noted degradation pattern of Basic Violet 3 [26]. This determination would be difficult considering that even pure Basic Violet 3 samples contain varying concentrations of Basic Violet 1. The ratio of BV3:BV1 in a pure sample can be affected anywhere along the process from synthesis to being mixed with other parts to make ink. As shown in this research, even a common desk lamp degrades the dyes in a black ballpoint ink in 72 hours to the extent that no 372 m/z or 358 ions are observed in the samples by mass spectrum. There is still no reliable way to correlate a sample's mass spectral signature and account for the plethora of variables in play once the ink leaves the pen tip and is deposited on paper. Once the line is written, volatiles enter the vapor phase, dyes can degrade, and other contaminants can be introduced to the document.

The ratio of BV3:BV1 is unstable and inconsistent, even within a contiguous ink line. In addition, the potential interferences mentioned present challenges to ink analysis. The ratio of BV3:BV1 is at best an indication that degradation has occurred in some BV 3 molecules to remove one methyl group or some BV 1 was initially present.

All black BP inks investigated displayed in their direct infusion ESI-MS spectra some peaks associated with known dye standards, such as BV 1, BV 3, and some contained Acid Yellow 36. The Basic dyes contained in BP inks were detectable with no additive in both the positive and negative operating polarity modes. These dyes that comprise such a substantial portion of these inks formulations are present in the highest detectable concentrations of all the components.

Ideally, it should be possible to identify an ink by comparing a sample extract to determine origin of the sample. After mass spectral analysis, simply coding a number "1" for an ion's presence above 3% relative intensity and a number "0" for no presence at or below 3%

relative intensity allows a discrimination table, like Figure 31, to be created. Once these table are created for different samples, they can be used to compare to other inks. The comparison consists of evaluating the binary code for m/z values where number “1’s” exist. The binary code from Method 1 is preferred as the starting point of the total evaluation of similarity. The reason for this is that it provides the most intense signal and can positively identify the dyes and some solvents components. If a sample matches a previously recorded Method 1 fingerprint, it would next have its Method 2 generated binary code compared. Method 3 is used similarly, except the value from the instrumental data that generates a binary code number “1” is 9% relative intensity. Method 3 uses negative applied polarity and typically generates more noise. A sample binary code can also be compared to standards stored in an online database.

CHAPTER FIVE: CONCLUSIONS AND FUTURE WORKS

The most common or top selling 100 inks (in BP, Gel, and RB classes) could be studied to see if a pattern emerges in their natural aging processes. For instance, the effect of a week's worth of sun on most cheaply made ink on a piece of paper when using mass spectral methods could be investigated and perhaps modeled. Other environmental effects (i.e. temperature, humidity, etc.) could also be studied. These variables affect the mass spectrum in ways that could create false negatives. An algorithm should be investigated for fitness as a model for BP ink aging trends. Gel and RB ink aging also creates issues in identification of their source. The volatiles in gels are the viscous glycols and are slightly more persistent than ethanol, or IPA. RB inks can contain dyes, pigments or a combination of the two. The trouble with this is that if we had a sample that was an RB with Basic Violet 3 as a coloring agent and it also has carbon black pigments, after some period of exposure to air, paper, and light the BV3 may completely degrade into smaller molecules like phenol. Without the 372 or 358 m/z peak the method would not identify BV3's presence. Once the methyl groups are cleaved a new compound is formed, if BV3 (372 m/z) has one methyl group cleaved it is degraded in to BV 1 (358 m/z) by some mechanism.

The plastic body of the pen may read "Bic Round Stic," however the actual portions of the formulation that are deposited on the paper are even unknown. The variation of the fingerprint based on composition of the ink at the time of deposition can lead to significant risk of misinterpretation. There is also the variation of formula known as "batch to batch" variation due to manufacturer purchase the raw materials from a non-consistent supply source. Ink formulation chemists would have data on how they mixed their batches, so they can, calculate costs. For purposes of keeping track of these variations, it would be easier and cheaper for the

ink industry to keep records of mass spectral data gathered with use of a method such as the direct infusion ESI-MS rather than using Rare Earth taggants. Testing for the Rare Earth taggants may be looking for a single or perhaps, a combination of several chemicals in known concentrations and known ratios to other ink components. However, ICP-MS is expensive and typically not used in a majority of forensic labs.

Since the direct infusion ESI-MS methods generate a fingerprint spectrum, an analyst does not need to know the definitive ID of every single ion or peak above 3% relative abundance in a sample's mass spectrum. The mass spectrum of some inks, like gels, can become quite complex and include adducts, degradation products, and polymers. Tables 7, 8 and 9 compare ions observed with each ink sample. Among the more vexing challenges presented for analysis of ink are the aging of ink-on-paper samples.

The results of this study indicate that with the direct infusion ESI-MS method detailed, a majority of the compounds are not contained within the fibers in detectable amounts soon after deposition. This appears to contradict previous studies based on a comparison of extraction into a so-called "weak" solvent like methanol and a "strong" solvent like trichloroethane followed by subsequent thin layer chromatographic detection procedure.

The most common or top selling 100 inks (in BP, Gel, and RB classes) could be studied to see if a pattern emerges in their natural aging processes. For instance, the effect of a week's worth of sun on most cheaply made ink on a piece of paper when using mass spectral methods could be investigated and perhaps modeled. Other environmental effects (i.e. temperature, humidity, etc.) could also be studied. These variables affect the mass spectrum in ways that could create false negatives. An algorithm should be investigated for fitness as a model for BP ink aging trends. Gel and RB ink aging also creates issues in identification of their source. The

volatiles in gels are the viscous glycols and are slightly more persistent than ethanol, or IPA. RB inks can contain dyes, pigments or a combination of the two. The trouble with this is that if we had a sample that was an RB with Basic Violet 3 as a coloring agent and it also has carbon black pigments, after some period of exposure to air, paper, and light the BV3 may completely degrade into smaller molecules like phenol. Without the 372 or 358 m/z peak the method would not identify BV3's presence. Once the methyl groups are cleaved a new compound is formed, if BV3 (372 m/z) has one methyl group cleaved it is degraded in to BV 1 (358 m/z) by some mechanism.

Mass spectra collected using direct infusion ESI-MS methods and careful spectral subtractions to elicit a relatively "pure ink'' spectrum (Figure 15D) with all three analytical methods. A proposed method of positive identification is an additive chemical placed in all inks that is able to form a product at a detectable level. This product should provide a uniform rate of decay or formation no matter what the storage conditions may be. Perhaps something similar to povidone in pharmaceuticals, which in preparations such as Betadine releases iodine in a predictable manner, could be utilized. Polymers that degrade in a well- understood manner with detectable degradation products or leave a chemical marker in a time correlated concentration. The use of the rare earth taggants by ICP-MS is not feasible, as rare earth compounds are usually expensive and the instrumentation is uncommon in a majority of labs. Perhaps an enzyme that degrades a substrate added to ink only as a marker of age. If the analyst has a clear picture of a document's age, this can be taken in to account when the report is issued. An automated online accessible database could be implemented based on data gathered using this method. The use of a search algorithm using clustering or another unsupervised pattern recognition technique could speed up the identification process. Any database created would be a subset of the data range of

the mass spectra of every ink. This is not only impossible, but it isn't feasible to even attempt to do anything other than develop a representative sample of assigned classes of inks.

One area that could be improved with the previously described direct injection ESI-MS analysis protocol would be to use a mass spectrometer that can switch analysis polarities during analysis. If the polarity of the instrument could be changed during an analytical run one or more of those methods could be combined and less samples would be consumed as a result. Use of a tandem mass spectrometer with direct infusion ESI-MS protocol would help achieve a definitive identification of ink components.

The way to possibly differentiate these samples further is to add to the database the sample FTIR. Also, the polarized light behavior of the sample could be coded to create a comparison score to improve discrimination. The comparison score allows for a quick check of samples that are close enough to merit further investigation. Ultraviolet spectra from inks may be a difficult element for a comparison, as they are not specific enough to allow differentiation of similarly colored inks.

The collaboration of efforts of all groups is vital to the ability to categorize the group of items called “ink.” A free flow of information, ease of access/use, and simplicity are the best ways to encourage more participation from groups who are not law enforcement. The effect of storage conditions might be more easily grasped if one hundred analysts gathered data in a central location and used standardized methods analysis so the comparisons, deductions, interpretations, inductions, extrapolations, interpolations are founded on the same scale.

If this methodology is employed on a working basis and the database is maintained and updated with new data, conclusions based on its results become more reliable. A system of proficiency testing from remote laboratories with web form input and instant analysis by

programs residing on the database server could be implemented. Analysts and laboratories could become instructed and certified on which ever method is used. Then individual variation due to the operator technique and, instrumental noise could be limited.

LIST OF REFERENCES

1. Saferstein, R., Forensic Science Handbook. 1982, Prentice-Hall: Englewood Cliffs, N.J. p. v. <1-3 >.
2. Williams, M.R., Moody, M., Arceneaux, L.E., Rinke, C., White, K., Sigman, M.E., Analysis of black writing ink by electrospray ionization mass spectrometry. Forensic Science International. In Press, Corrected Proof.
3. Gernandt, M.N. and J.J. Urlaub, An introduction to the gel pen. Journal of Forensic Sciences, 1996. 41(3): p. 503-504.
4. Mazzella, W.D. and A. Khanmy-Vital, A study to investigate the evidential value of blue gel pen inks. Journal of Forensic Sciences, 2003. 48(2): p. 419-424.
5. Hilton, O., Scientific examination of questioned documents. 1993, Boca Raton, FL: CRC Press. xv, 424 p.
6. El-Din, N.M.S., M.W. Sabaa, and H.R. Hamed, Influence of Three Chemical Erasures on Different Types of Inks Marked on Several Document Papers: An Analysis of the Chemical Reactions Occurring on Paper Between Different Liquid Erasures and Writing Inks. International Journal of Forensic Document Examiners, 1998. 4(2): p. 9.
7. Godown, L., New nondestructive document testing methods. Journal of Criminal Law and Criminology, 1964. 55: p. 280.
8. Derek, L.H., Validation of LAB Color Mode as a Nondestructive Method to Differentiate Black Ballpoint Pen Inks*. Journal of Forensic Sciences, 2007. 52(4): p. 967-973.
9. Brunelle, R.L. and K.R. Crawford, Advances in the forensic analysis and dating of writing ink. 2003, Springfield, Ill.: Charles C Thomas. xix, 215 p.

10. Brunelle, R.L. and K.R. Crawford, Advances in the forensic analysis and dating of writing ink. 2003, Charles C. Thomas, Publisher Ltd.

11. Zlotnick, J.A. and F.P. Smith, Chromatographic and electrophoretic approaches in ink analysis. Journal of Chromatography B: Biomedical Sciences and Applications, 1999. 733(1-2): p. 265-272.

12. Holmes, J.L., C. Aubry, and P.M. Mayer, Assigning structures to ions in mass spectrometry. 2007: CRC Press.

13. LaPorte, G.M., et al., The Identification of 2-Phenoxyethanol in Ballpoint Inks Using Gas Chromatography/Mass Spectrometry-Relevance to Ink Dating. Journal of Forensic Sciences, 2004. 49(1): p. 155-159.

14. Grim, D.M., J. Siegel, and J. Allison, Does ink age inside of a pen cartridge? Journal of Forensic Sciences, 2002. 47(6): p. 1294-1297.

15. Ng, L.K., P. Lafontaine, and L. Brazeau, Ballpoint pen inks: characterization by positive and negative ion-electrospray ionization mass spectrometry for the forensic examination of writing inks. Journal of Forensic Sciences, 2002. 47(6): p. 1238–1247.

16. Abian, J., The coupling of gas and liquid chromatography with mass spectrometry. Journal of Mass Spectrometry, 1999. 34: p. 157-168.

17. Herbert, C.G. and R.A.W. Johnstone, Mass spectrometry basics. 2002: CRC.

18. Huang, M., J. Yinon, and M.E. Sigman, Forensic identification of dyes extracted from textile fibers by liquid chromatography mass spectrometry (LC-MS). Journal of Forensic Sciences, 2004. 49(2): p. 238-249.

19. Temesl, D. and B. Law, The effect of LC eluent composition on MS responses using electrospray ionization. LC GC, 1999. 17(7): p. 626-632.

20. Lauren, M.P., A.W. Trevor, and W.R. Fawcett, High-Performance Liquid Chromatography; Ultraviolet; Visible Spectroscopy; Electrospray Ionization Mass Spectrometry Method for Acrylic and Polyester Forensic Fiber Dye Analysis. Journal of Forensic Sciences, 2006. 51(4): p. 771-779.

21. Lociciro, S., et al., Dynamic of the ageing of ballpoint pen inks: quantification of phenoxyethanol by GC-MS. Science & Justice, 2004. 44(3): p. 165-171.

22. Weyermann, C., et al., Evaluation of the Photodegradation of Crystal Violet upon Light Exposure by Mass Spectrometric and Spectroscopic Methods. Journal of Forensic Sciences, 2009. 54(2): p. 339-345.

23. Denman, S., et al., Photostability of Crystal Violet (CI 42555). Dyes and Pigments, 1996. 30(1): p. 67-72.

24. Andrasko, J., Changes in composition of ballpoint pen inks on aging in darkness. Journal of Forensic Sciences, 2002. 47(2): p. 324.

25. Egan, J.M., K.A. Hagan, and J.D. Brewer, Forensic analysis of black ballpoint pen inks using capillary electrophoresis. Forensic Science Communications, 2005. 7(3).

26. Jamie, D.D. and A. John, The Detection of Multiply Charged Dyes Using Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry for the Forensic Examination of Pen Ink Dyes Directly from Paper. Journal of Forensic Sciences, 2007. 52(5): p. 1205-1211.

27. LaPorte, G.M., et al., An Evaluation of Matching Unknown Writing Inks with the United States International Ink Library*. Journal of Forensic Sciences, 2006. 51(3): p. 689-692.

28. The National Academies Press. Strengthening Forensic Science in the United States: A Path Forward. http://www.nap.edu/catalog.php?record_id=12589#toc