Determination of Arsenic Species in Electronic Cigarette Liquid

by

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Abstract

The presence of arsenic in e-liquids of electronic cigarettes has rarely been studied and the few studies that have investigated the presence of arsenic have found either very low, or undetectable concentrations. Additionally, the individual species of arsenic have not been thoroughly explored due to the viscosity of the sample matrix. The focus of this research was to determine both the total arsenic concentration and concentrations of individual arsenic species present in e-liquids. A total of 96 e-liquid samples were purchased from three online vendors and selected based on popularity. For the determination of total arsenic concentrations, inductively coupled plasma mass spectrometry (ICP-MS) was used. Arsenic was detected in 81 of 96 e-liquid samples with most (62/81) having concentrations under 3 μg/kg. Only eight e-liquids had elevated arsenic concentrations greater than 5 μg/kg, with five having concentrations between 16 and 36 μg/kg. Due to the multi-element capability of ICP-MS, fourteen other elements including antimony, cadmium, chromium, cobalt, and lead were also determined. Arsenic species were investigated using ion pair and anion exchange high performance liquid chromatography (HPLC) separation with ICP-MS detection, however arsenic species concentrations were only determined using anion exchange HPLC-ICP-MS. Four arsenic species; arsenite (As^{III}), dimethylarsinic acid (DMA), monomethylarsonic acid (MMA) and arsenate (As^{V}) were determined in the eight e-liquids with arsenic concentrations higher than 5 μg/kg. Both As^{V} and As^{III} were present in all eight samples, with As^{V} having the highest concentration in seven of the eight samples with a median concentration of 4.42 μg/kg, followed by As^{III} which had a median concentration of 1.72 μg/kg. Inorganic arsenic (As^{III} + As^{V}) concentrations in the eight e-liquids were between 3.6 μg/kg to 16.3 μg/kg and from these e-liquids inorganic arsenic concentrations in the air were estimated to be between 2.7 μg/m^3 to 12.2 μg/m^3. When compared to the PEL set by OSHA of 10 μg/m^3, the upper range of 12.2 μg/m^3 is greater, meaning that if an e-cigarette user were to vape for an eight-hour period they could be exposing themselves to inorganic arsenic concentrations in the air greater than the recommended PEL set by OSHA. Four unknown arsenic species were also detected in 50% - 88% of the samples analyzed highlighting the need for additional research to determine the identity of these unknown arsenic species.

Preface

This thesis is original work done by Jordan Schofield. No part of this thesis has been previously published.

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NH_4HCO_3	Ammonium bicarbonate
$^{40}Ar^{35}Cl^+$	argon chloride
AsH_3	arsine or arsenic hydride
As^V	arsenate
As^{III}	arsenite
AsB	arsenobetaine
AsC	arsenocholine
AAS	atomic absorption spectrometry
AFS	atomic fluorescence spectrometry
CRM	certified reference material
DMA	dimethylarsinic acid
e-cigarettes	electronic cigarettes
e-liquids	electronic liquids
EPA	Environmental Protection Agency
ESI-MS	electrospray ionization mass spectrometry
HPLC	high performance liquid chromatography
HG-ICP-MS	hydride generation ICP-MS
HCl	hydrochloric acid
ICP	inductively coupled plasma
ICP-MS	inductively coupled plasma mass spectrometry
KED	kinetic energy discrimination
MDL	method detection limit
MMA	monomethylarsonic acid
HNO_3	nitric acid
OxoAsS	Oxo-arsenosugars
PEL	permissible exposure limit
PAH	polycyclic aromatic hydrocarbon
PG	propylene glycol
$NaBH_4$	sodium borohydride
SRM	standard reference material

H_2SO_4	sulphuric acid
TBA	tetrabutylammonium hydroxide
TMA	tetra methylarsonium ion
thioAsS	Thio-arsenosugars
TMAO	trimethylarsine oxide
OSHA	United States Occupational Safety and Health Administration
VG	vegetable glycerin
VOC	volatile organic compounds
WHO	World Health Organization

Chapter 1: **Introduction**

1.1 E-cigarettes

Electronic cigarettes (e-cigarettes) are devices that are used to inhale an aerosolized liquid via a mouthpiece. There are numerous kinds of e-cigarettes on the market with a wide array of appearances. Some e-cigarettes can resemble combustible tobacco cigarettes while others can look like everyday items such as USB sticks and pens. In general, e-cigarettes are composed of a mouthpiece, a tank or cartridge for holding the e-liquid, a heating coil and atomizer where the e-liquid is aerosolized, and a battery.

The first e-cig was invented in the mid 2000's (National Academies of Sciences, Engineering and Medicine, 2018; Deng et al., 2020) and since then there have been three generations of e-cigarettes. First generation e-cigarettes closely resembled combustible tobacco cigarettes, second generation e-cigarettes were larger than the first generation, and had a clear transparent cartridge/tank, and third generation e-cigarettes often do not resemble tobacco cigarettes and are customizable.

E-cigarettes were designed, marketed, and advertised to be a less harmful alternative to combustible tobacco products as well as another tool to aid in smoking cessation. However, it is inconclusive whether e-cigarettes are an effective way to quit smoking. Furthermore, the general public are often misled by these advertisements, particularly youth and young adults (Gentzke et al., 2019; Deng et al., 2020). The use of e-cigarettes among youth in the United States increased from 1.5% to over 20% between 2011-2018 (Gentzke et al., 2019).

Like combustible cigarettes, e-cigarettes are used to deliver nicotine into the body. This is accomplished by using a solution known as e-liquid (also referred to as e-fluid, e-juice, vape juice etc.) which is aerosolized and then inhaled by the user. In addition to nicotine, other substances have been added to e-liquids such as flavouring, pharmaceuticals, and illicit drugs etc. (National Academies of Sciences, Engineering and Medicine, 2018).

E-liquid is typically composed of delivery solvents, flavouring, and an optional amount of nicotine. The delivery solvents are typically propylene glycol (PG) and vegetable glycerin (VG). However, other delivery solvents, such as ethylene glycol, have also been used (Hutzler et al., 2014). The delivery solvents, PG and VG, are commonly used to make e-liquids and are used at various ratios, for example 30 PG:70 VG or 50 PG:50 VG. Due to the viscosity of these delivery solvents, among other factors, certain delivery solvent ratios are recommended for one type of e-cigarette device over another.

When it comes to flavouring there are numerous different flavours of e-liquids on the market. Research done by Zhu and colleagues in 2014 found over 7700 flavours in 466 brands studied (Zhu et al., 2014). Flavouring in e-liquids are very common and considered to be an important deciding factor for e-liquid use and consumption (National Academies of Sciences, Engineering and Medicine, 2018).

The nicotine used in e-liquids can be classified into two categories; free base nicotine, which is the same form that is present in combustible cigarettes, and nicotine salts which are the natural form of nicotine found in tobacco leaves. E-liquids that are made with free base nicotine typically have lower nicotine concentrations whereas e-liquids that are made with nicotine salts can contain higher concentrations of nicotine (Barrington-Trimis & Leventhal, 2018; Kechter et al., 2021)

In Canada e-cigarette products have been regulated under various provincial acts since 2015 but were not federally regulated until May 2018. The federal regulations include the Tobacco and Vaping Products Act, Non-smokers' Health Act, and Canada Consumer Product Safety Act (*Tobacco Legislation*, 2021).

In addition to nicotine, flavourings, and solvent carriers (PG and VG), other substances and compounds that are known to cause various health effects, such as polycyclic aromatic hydrocarbons (PAH), volatile organic compounds (VOC), metals, metalloids, and drugs, have been identified in e-liquids and aerosols (National Academies of Sciences, Engineering and Medicine, 2018).

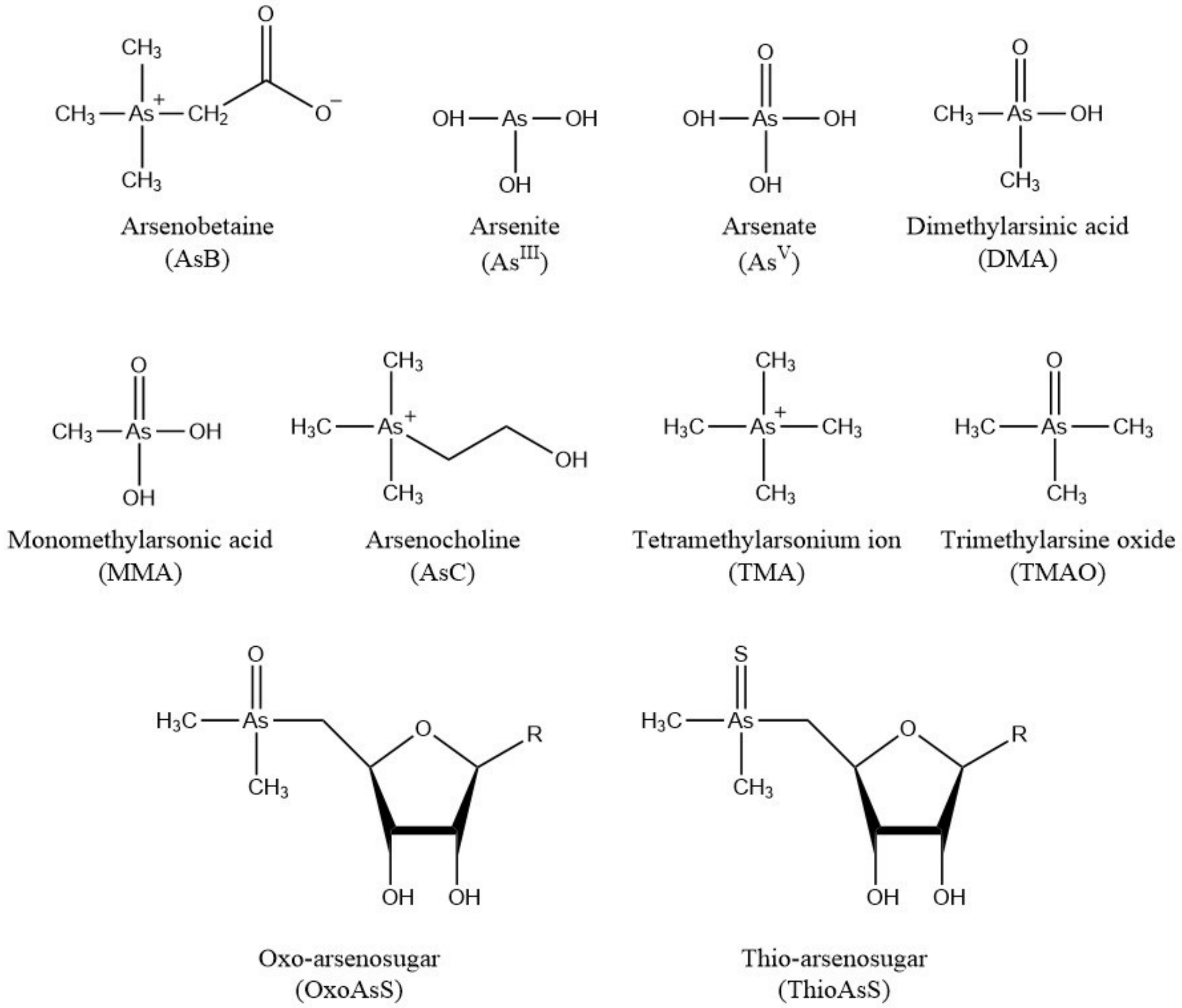
1.2 Arsenic

1.2.1 Overview & Background

Arsenic is a naturally occurring metalloid found throughout the earth's crust and subsequently, the environment. The main sources of arsenic exposure by the general public are drinking water and food, specifically aquatic foods (eg fish) and plants. The World Health Organization (WHO) estimates that at least 140 million people around the world are exposed to high concentrations of arsenic via drinking water. While many countries meet the WHO guidelines for arsenic in drinking water, which is set at 10 µg/L, due to various factors not all countries can meet this guideline, such as Bangladesh, Argentina, Vietnam, and Mexico (WHO, 2018). The United States Occupational Safety and Health Administration (OSHA) as set a permissible exposure limit of inorganic arsenic in the air of 0.010 mg/m^3 or 10 $µg/m^3$ over an eight-hour period (US NIOSH, 1988). Due to the toxicity of inorganic arsenic, human exposure remains a global health concern (Reid et al., 2020) and long term exposure to arsenic has been known to cause various adverse health effects such as cancers, cardiovascular, and neurological diseases (Hughes et al., 2011; Khan et al., 2020)

It has been well documented that arsenic can exist as many different species, and that the toxicity of these species depends on both its chemical form and oxidation state (Reid et al., 2020). For example, As^{III} is a highly toxic form of arsenic while arsenobetaine (AsB) is considered virtually non-toxic (Styblo. et al., 2000; Moe et al., 2016; Jia et al., 2018). Figure 1 shows some of the common arsenic species that have been reported.

Arsenic speciation is commonly done using HPLC separation with ICP-MS detection (Reid et al., 2020; Sadee et al., 2015). Separation of individual arsenic species is vital for their quantification and identification, and the various modes of HPLC, such as anion exchange and cation exchange, can effectively separate many arsenic species. Other spectrometric techniques have been used for the detection of arsenic species including atomic absorption spectrometry (AAS), atomic fluorescence spectrometry (AFS), and inductively coupled plasma optical emission spectrometry (Zou et al., 2019). However, ICP-MS remains the most common detection method for arsenic because of its high ionization efficiency, low matrix interference, high selectivity and high sensitivity (Chan & Caruso, 2012; Leermakers et al., 2006; Luvonga et al., 2020; Montes-Bayón et al., 2003; Reid et al., 2020; Zou et al., 2019). Despite these advantages ICP-MS does not give any molecular information and thus arsenic species identification relies on matching retention times with available standards.



Arsenobetaine (AsB)

Arsenite (As^{III})

Arsenate (As^{V})

Dimethylarsinic acid (DMA)

Monomethylarsonic acid (MMA)

Arsenocholine (AsC)

Tetramethylarsonium ion (TMA)

Trimethylarsine oxide (TMAO)

Oxo-arsenosugar (OxoAsS)

Thio-arsenosugar (ThioAsS)

Figure 1.2. Names and structures of commonly reported arsenic species

1.2.2 Total arsenic analysis

As previously mentioned, ICP-MS is the most common detection method for arsenic analysis because of its high ionization efficiency, low matrix interference, high selectivity, and high sensitivity (Chan & Caruso, 2012; Leermakers et al., 2006; Luvonga et al., 2020; Montes-Bayón et al., 2003; Reid et al., 2020; Zou et al., 2019). In general, when determining total arsenic content, solid samples are typically digested in acid solutions amenable for ICP-MS analysis. Acid digestion usually involves either nitric acid (HNO_3), sulphuric acid (H_2SO_4) or a combination of both (Gupta et al., 2018; Liu et al., 2015; Peng et al., 2014). Additionally, acid digestions converts all the arsenic species present in the sample into one form, specifically inorganic arsenic. Hydrochloric acid (HCl) is usually avoided due to increased polyatomic interferences, such as argon chloride ($^{40}Ar^{35}Cl^+$).

Depending on the sample type, such as fish or chicken tissue, microwave-assisted digestion may be used to ensure complete digestion of the sample as well as conversion of arsenic species into their inorganic form (Gupta et al., 2018). Other sample types, such as urine or water, usually only need to be acidified and do not require the same acid digestion procedure as fish or chicken tissue (Chen et al., 2010; Kippler et al., 2016).

When it comes to total arsenic analysis for e-liquids and aerosols, these samples are prepared in a similar fashion as water or urine samples. Additionally, because of the high viscosity of the e-liquids, these samples are diluted with either de-ionized water (Olmedo et al., 2018) or diluted nitric acid (Beauval et al., 2016; Palazzolo et al., 2017) to minimize possible matrix effects.

1.2.3 Arsenic speciation analysis

When choosing a method for arsenic speciation analysis, it is important to consider how the sample preparation will affect and interact with both the physiochemical properties of the arsenic species of interest and the sample matrix. The chosen preparation method should have both high extraction efficiency for the arsenic species of interest and should prevent or minimize any interconversion between arsenic species. Depending on the species of interest, and the type of sample, choosing such a method can be challenging.

To prevent or minimize arsenic species interconversion, mild extraction methods are used. Common mild extraction solvents, used to extract polar arsenic species, include methanol-water, acetonitrile-water, and dilute phosphoric acid. Different ratios of methanol-water have been shown to affect extraction efficiency of arsenic species in rice, chicken, fish and soil samples (Pizarro et al., 2003). Due to the use of mild extraction conditions, and the type of sample being investigated, multiple extractions may be needed to maximize extraction efficiency. When detecting trace amounts of arsenic species, preconcentration of extracts before analysis may be required.

Despite multiple extractions and preconcentration, mild extraction solvents like methanol-water can suffer from low extraction efficiency when applied to solid samples that contain proteins such as fish or chicken tissue. For example work done by Zhao et al. compared extraction efficiencies of various organic, aqueous and acidic solvents, in addition to three enzymes, on fish tissue (Zhao et al., 2018). The authors found that for the eleven arsenic species investigated, the use of enzymes, specifically protease, achieved an extraction efficiency of 94% - 106%.

Work done by Liu et al. compared the extraction efficiencies of methanol-water at a 1:1 ratio against various enzymes on chicken meat samples (Liu et al., 2015). The authors demonstrated that the methanol-water extraction only had a 28% extraction efficiency while the use of enzymes, specifically papain, in conjunction with sonication increased the extraction efficiency to 88%. Peng et al. also demonstrated that the use of pepsin resulted in an extraction efficiency of 83% - 93% of arsenic species in chicken liver (Peng et al., 2014).

When it comes to liquid samples such as water or urine the sample preparation is less intensive. These types of samples typically only require filtration before analysis but can also require dilution if necessary (Chen et al., 2010; Rodríguez et al., 2021; Scheer et al., 2012; R. Xie et al., 2006). E-liquids can be prepared in a similar way as water or urine samples for arsenic speciation analysis. To date there has only been one study that has investigated arsenic species in e-liquids, and it was demonstrated by Liu et al. that dilution and filtration was sufficient sample preparation for the analysis of e-liquids (Liu et al., 2020).

1.3 ICP-MS

ICP-MS was first introduced over 30 years ago and has become a popular detection technique and has been applied to a variety of different samples (Barnet et al., 2021; Contreras-Acuña et al., 2014; Peng et al., 2014; Yang et al., 2020; Zhao et al., 2018; Zou et al., 2019). ICP-MS is the most common detection method for arsenic analysis because of its low matrix interference, high selectivity, and high sensitivity (Chan & Caruso, 2012; Leermakers et al., 2006; Luvonga et al., 2020; Montes-Bayón et al., 2003; Reid et al., 2020; Zou et al., 2019).

There are several components that make up an ICP-MS instrument. Some of the key components include a sample introduction system, inductively coupled plasma (ICP), mass analyzer, and detector. The sample introduction system includes a nebulizer and spray chamber. Liquid samples are nebulized into a fine aerosol which is then carried to the ICP torch via argon gas. As the aerosol passes through the torch, elements undergo atomization and ionization in the high temperature argon plasma. Ions in the gas phase are then extracted through an interface region by electrostatic lenses and being focused and guided into the mass analyzer. The mass analyzer then separates ions based on their respective mass-to-charge ratio (m/z) which are then recorded by the detector. Optionally, a collision cell can be installed before the mass analyzer to reduce polyatomic interferences.

Some advantages that ICP-MS has over other instruments such as AAS or AFS includes simultaneous multi-element detection and isotopic analysis (Gong et al., 2002; Montes-Bayón et al., 2003) in addition to detection limits that can reach sub part per billion levels depending on the method and sample (Liu et al., 2015, 2020; Peng et al., 2014; Vassileva et al., 2001).

For arsenic speciation analysis, both sensitive detection and efficient separation are critical which is why ICP-MS is commonly coupled to HPLC. This coupling takes advantage of the sensitive detection of ICP-MS and the efficient separation of HPLC, in addition to both instruments being easily compatible with one another.

Hydride Generation ICP-MS (HG-ICP-MS) is another technique that has been employed for arsenic speciation analysis (Chen et al., 2010; Pétursdóttir & Gunnlaugsdóttir, 2019). Eluent from the HPLC is put through a hydride generation system where an acid (HNO_3, HCl) and sodium borohydride ($NaBH_4$) are continuously added. The arsenic hydride, or arsine (AsH_3) product generated from this process is then detected by the ICP-MS. The advantage of this technique is that AsB, which does not react with $NaBH_4$, can be discriminated from other hydride-forming arsenicals such as inorganic arsenic or its methylated metabolites. This technique can be very useful when looking at samples that contain both AsB and As^{III}.

While ICP-MS can provide great sensitivity, selectivity, and low detection limits, it cannot provide any molecular information. Therefore, identification of arsenic species using HPLC-ICP-MS relies on matching retention time information with available standards. It is for this reason that HPLC-ICP-MS cannot identify compounds without the use of standards or reference materials and that co-eluting peaks cannot be distinguished.

Additionally, ICP-MS can suffer from polyatomic interferences, such as $^{40}Ar^{35}Cl^+$ (m/z 75), when samples contain chloride. This can be mitigated using kinetic energy discrimination (KED) and a collision cell where reaction/collision gas such as helium or hydrogen, is introduced. Samples ions enter this cell before moving onto the mass analyzer and collide with the reaction/collision gas. Because polyatomic ions, such as $^{40}Ar^{35}Cl^+$, are larger than analyte ions, such as As^+, they undergo more collisions with the collision gas. Due to the loss of kinetic energy, these polyatomic interferences are unable to reach the detector.

1.4 HPLC

HPLC is a chromatographic separation technique used for separating molecules of interest in a liquid sample. The sample is mixed with a solvent, or mobile phase, and is carried through a column that is packed with a stationary phase where analytes are separated based on their partitioning between the stationary and mobile phases.

Chromatography was first used by Mikhail Tswett in the early 1900's while studying the adsorption of leaf pigments. It wasn't until the second half of the 1900's when high pressure liquid chromatography was first used and since then has become one of the major separation techniques used worldwide. There are a variety of different modes or types of HPLC. For arsenic analysis, the most common HPLC modes include anion exchange, cation exchange, and reverse phase.

In anion exchange chromatography, arsenic species are separated based on their respective pKa values using a positively charged stationary phase. The positively charged stationary phase is usually composed of nitrogen containing compounds such as quaternary ammonium [$-N^+(CH_3)_3$] functional groups which are bonded to either a polymer or silica backbone. The most common anionic arsenic species include As^V, As^{III}, MMA, and DMA. Other species, such as As^{III}, AsB, arsenocholine (AsC), oxo-arsenosugars (OxoAsS), thio-arsenosugars (thioAsS), and phenylarsenicals have also been separated using anion exchange chromatography (Liu et al., 2018; Reid et al., 2020). The typical elution order when using a strong anion exchange column is AsB, As^{III}, DMA, MMA and As^V, which is consistent with their respective pKa values. Because AsB is a zwitter ion, and As^{III} has a high pKa (9.2), these two species are not well retained in an anion exchange column. A good strategy to separate these two species is to increase the pH of the mobile phase, thus increasing the fraction of deprotonated As^{III} resulting in an increase of retention on As^{III}.

In cation exchange chromatography, positively charged arsenic species such as AsB, AsC, trimethylarsine oxide (TMAO), and tetra methylarsonium ion (TMA) are separated using a strong cation exchange column that has a negatively charged stationary phase (Reid et al., 2020). The negatively charged stationary phase usually contains sulfate, sulfonate, or carbonate groups. Here the stronger positively charged species are retained more in the column.

Reverse phase chromatography is another mode of HPLC that is used for arsenic speciation analysis. In general, this mode of HPLC separates molecules based on their polarities and is useful for the analysis of arsenolipids, which are a broad class of arsenic containing compounds that include hydrocarbons, fatty acids, fatty alcohols, and phospholipids (Taylor et al., 2017). Arsenosugars, which are arsenic contain sugar compounds, have also been separated using reverse phase chromatography (Raab et al., 2013) . A C_8 or C_{18} column is typically used for analyzing arsenolipids and arsenosugars as it enables these species to be separated based on what functional groups are present, number of double bonds, and the number of carbon atoms present.

Ion pair chromatography, like reverse phase, uses the same C_8 or C_{18} columns but has ion paring reagents added to the mobile phase which allows for the separation of ionic and neutral arsenic species (Afton et al., 2008; Kaňa et al., 2020; Nan et al., 2018). Ion pair reagents are comprised of a charged region that interacts with the analytes, and a hydrophobic region that interacts with the stationary phase of the column. For separating anionic and neutral arsenic species, tetraalkylammonium, tetrabutylammonium and tetraethylammonium are the ion pair reagents typically used (Reid et al., 2020). When separating cationic and neutral arsenic species, alkyl sulfonates such as hexanesulfonic acid and 1-pentane sulfonic acid are the ion pair reagents used (Reid et al., 2020).

1.5 Rationale and objectives of the thesis research

This research focusses on the study of arsenic in e-liquids. E-liquids were investigated because they are marketed as a less harmful alternative to combustible tobacco cigarettes and are relatively new to consumers. Moreover, their long-term health effects are not fully understood due to their limited time on the market. To date in the literature, there has only been one study that has investigated arsenic species in e-liquids (Liu et al., 2020) and in this study only 17 e-liquids were analyzed. By determining the total arsenic concentration, and the concentration of individual arsenic species, both regulators and consumers can be better informed on levels of contaminants, including arsenic, that are present in e-liquids. To date this research is the largest study in terms of e-liquid samples analyzed investigating various metals and metalloids in 96 samples and captures a wide breadth of representative e-liquids that have different PG/VG ratios and nicotine concentrations. In this study there were two main objectives:

Firstly, to determine total arsenic concentration in e-liquids using ICP-MS. Due to previous research that has reported low concentrations of arsenic, ICP-MS analysis was preferred due to its high detection sensitivity which is crucial for trace element analysis. Additionally, due to the multi-element capability of ICP-MS, concentrations of other contaminants of concern such as antimony, cadmium, chromium, cobalt, and lead, among others that have been reported in e-liquids, will be determined.

Secondly, determination of four common arsenic species present in e-liquids will be investigated. While ICP-MS provides highly sensitive detection, by itself it is unable to distinguish between arsenic species. However, when ICP-MS is combined with HPLC separation, individual arsenic species can now be distinguished. Two different modes of separation using ICP-MS for detection will be used to determine arsenic species present in e-liquids.

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Chapter 2: Determination of Multiple Elements in Electronic Cigarette Liquid

2.1 Introduction

Arsenic is a toxic element of concern that is present throughout the environment. Water and food are main sources of exposure for the general population (WHO, 2018). Long term exposure to arsenic can cause various adverse health effects such as cancers, cardiovascular diseases and neurological diseases (Hughes et al., 2011; Khan et al., 2020; WHO, 2018).

Various techniques have been used for the detection of arsenic including atomic absorption spectrometry (AAS), atomic fluorescence spectrometry (AFS), and inductively coupled plasma mass spectrometry (ICP-MS) (Zou et al., 2019). ICP-MS provides simultaneous multielement detection along with high sensitivity, low matrix interference, and high selectivity, making it the most common detection technique used for arsenic analysis (Luvonga et al., 2020; Reid et al., 2020).

E-cigarette use has risen over the last decade (Bao et al., 2020; Cerrai et al., 2020; Dai & Leventhal, 2019; Gorini et al., 2020; Walker et al., 2020) and while current research shows that e-cigarette aerosol contains lower amounts and lower concentrations of toxic substances when compared to combustible tobacco cigarettes, long term health effects are not well known (Groner, 2022; National Academies of Sciences, Engineering and Medicine, 2018; Re et al., 2021)

Previous studies on e-liquids have reported the presence of various contaminants including antimony, arsenic, chromium, lead, and nickel (Beauval et al., 2016; Mikheev et al., 2016; Olmedo et al., 2018, 2021; Zhao et al., 2019). However, due to the high viscosity of e-liquids, samples require large dilution factors to minimize possible matrix effects and avoid clogging the nebulizer or the interface cones. The large dilution factors used can also impact the number of trace elements reported.

In this chapter, ICP-MS was used for trace element analysis to determine the total concentration of arsenic along with fourteen other elements including antimony, cadmium, chromium, and lead in e-liquids.

2.2 Materials and Methods

2.2.1 Reagents and Standards

Deionized water from a Milli-Q purification system (18.2 MΩ, Millipore, Molsheim, France) was used throughout these experiments. Concentrated, optima grade nitric acid (HNO_3) from Fisher Scientific was diluted and used to acidify samples and standards. Calibration standards were freshly prepared each day from environmental calibration standard (Agilent Technologies, Santa Clara, U.S). Standard reference material (SRM) 1640a (trace elements in natural water) and SRM 1643f (trace elements in water) were obtained from the National Institute of Standards and Technology (Gaithersburg, MD, USA), and were used as quality control measures to ensure accurate total arsenic determination in addition to evaluating calibration curve accuracy.

2.2.2 Sample Analysis

E-liquid samples were purchased online from a variety of retailers which were selected based on having a high number of google reviews, and large inventories for sale. The samples were selected based on popular reviews on their respective websites. E-liquid sample bottles ranged from 30 mL to 100 mL in size and were stored at 4 °C until analysis. After inverting each bottle many times to ensure homogeneity, approximately 0.28-0.32 g of e-liquid sample was weighed into 15 mL polypropylene tubes and diluted twenty-fold with 2% HNO_3 to a volume of 5 mL, before being vortexed.

Calibration standards were made in the presence of e-liquid matrix. E-liquid matrix, matching the same PG/VG ratio in the samples to be analyzed, was diluted twenty times with 2% HNO_3 and was subsequently used in preparing the calibration standards. This solution was prepared fresh with each analysis. Calibration standards were prepared at the following concentrations (µg/L): 0.1, 0.2, 0.3, 0.4, 0.5, 1, 5, 10, 20 and 30 µg/L. The accuracy of these calibration standards was evaluated using SRM 1640a or 1643f which were also prepared fresh with each analysis.

SRM 1640a and 1643f were also prepared in the presence of matrix, matching the same PG/VG ratio in the samples to be analyzed. For SRM 1640a, approximately 0.58 – 0.61 g of e-liquid was weighed into a 15 mL polypropylene tube followed by the addition of 4.75 mL of SRM 1640a and 4.75 mL of 2% nitric acid. SRM 1643f was prepared in a similar fashion where similar amounts of e-liquid were weighed, followed by 1.75 mL of SRM 1643f and 7.75 mL of 2% nitric acid. In addition to the quality control samples mentioned above, a 5 µg/L and 30 µg/L standard was used as a check standard to check instrument drift, and was analyzed every 9 injections

The total concentration of arsenic along with fourteen other elements was determined using an ICP-MS (7900 series, Agilent Technologies, Santa Clara, U.S). Instrumental conditions are shown in Table 2.2 and were optimized before each analysis using a 1 µg/L Ce, Co, Li, Mg, Tl and Y in 2wt% HNO_3 tuning solution (Agilent Technologies, Santa Clara, U.S).

Table 2.2 Optimized ICP-MS parameters (Agilent 7900 system)

Plasma	
RF Power	1550 W
RF matching	1.70 V
Sampling depth	8.0 mm
Nebulizer gas	1.09 L/min
Nebulizer pump	0.30 rps
Spray chamber Temp	2 °C
Ion lenses	Optimized every run using a 1 µg/L Ce, Co, Li, Mg, Tl and Y in 2wt% HNO_3 tuning solution (Agilent Technologies, Santa Clara, U.S)
Collision cell	
He Flow	3.5 mL/min
OctP Bias	-18 V
OctP RF	200 V
Energy Discrimination	5 V
Data acquisition parameters	
Peak pattern	3 points
Integration time (per point)	0.1 s
Replicates	3

Method detection limits (MDL) were determined using the method described by the Environmental Protection Agency (EPA, 2017). Briefly, a series of spiked blanks (n = 9) were prepared at 0.2, 0.3, 0.5, 1, 5, and 10 µg/L. Standard deviations were calculated and multiplied by the Students' t-value at 99% confidence.

2.3 Results

Following the work done by Beauval et al., calibration curves were made in the presence of matrix corresponding with the PG:VG ratio found in samples being analyzed that day (Beauval et al., 2016). Figure 2.3.1 demonstrates how calibration curve slopes, a measure of analytical sensitivity, changed depending on the e-liquid matrix PG:VG added.

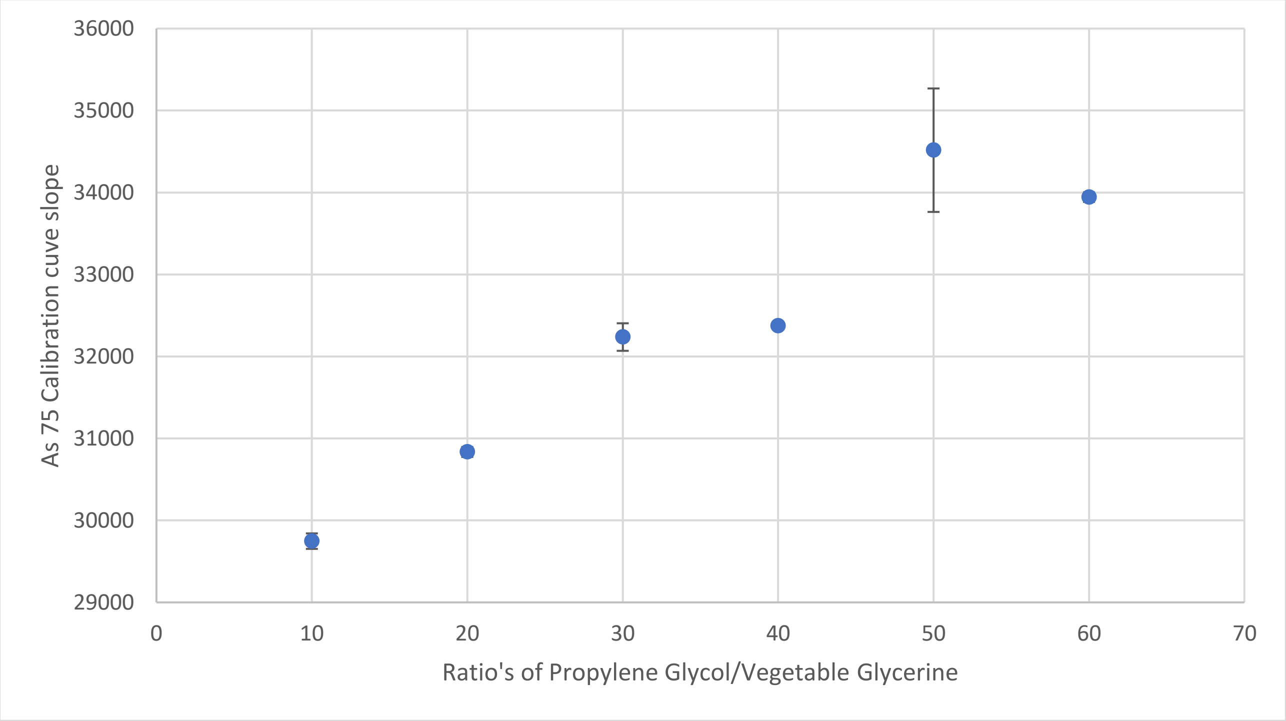

Figure 2.3.1 Various calibration curve slopes of total arsenic, made in the presence of different ratios of PG/VG e-liquid matrix (n = 3)

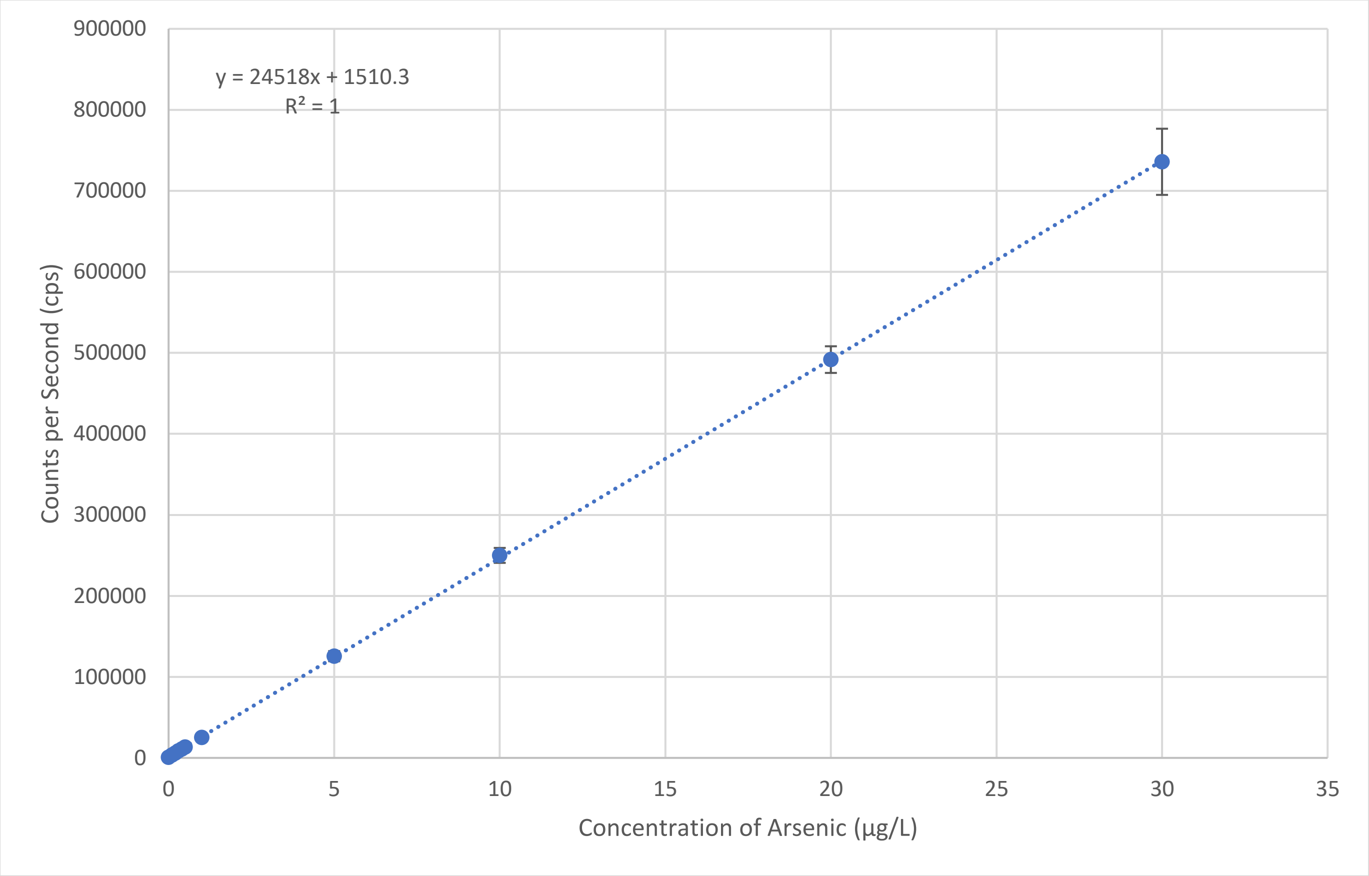

Fig 2.3.2 Representative Calibration curve generated from ICP-MS analysis of 0.1, 0.2, 0.5, 1, 5, 10, 20, and 30 µg/L arsenic standards (n = 3)

Table 2.3.1 Comparison of certified and measured concentrations of multiple elements in standard reference material SRM 1640a

Reference Material	Element of Interest	Certified Concentration (µg/L)	Measured Concentration (µg/L)*	Accuracy (%)
	Be 9	3.026 ± 0.0028	3.2 ± 0.4	105 ± 13
	Al 27	53.0 ± 1.8	59 ± 11	112 ± 20
	V 51	15.05 ± 0.25	16.5 ± 0.4	110 ± 2
	Cr 52	40.54 ± 0.3	41.9 ± 0.9	103 ± 2
	Mn 55	40.39 ± 0.36	44.5 ± 0.9	110 ± 2
	Co 59	20.24 ± 0.25	22.8 ± 0.6	113 ± 3
	Ni 60	25.32 ± 0.14	26.5 ± 0.4	105 ± 1
1640a	Cu 63	85.75 ± 0.51	87.8 ± 1.3	102 ± 2
	Zn 66	55.64 ± 0.35	60.2 ± 1.7	108 ± 3
	As 75	8.075 ± 0.07	8.27 ± 0.06	102 ± 1
	Ag 107	8.081 ± 0.046	8.4 ± 0.9	104 ± 11
	Cd 111	3.992 ± 0.074	4.22 ± 0.07	106 ± 2
	Sb 121	5.105 ± 0.046	5.57 ± 0.12	109 ± 2
	Tl 205	1.619 ± 0.016	1.80 ± 0.04	111 ± 3
	Pb 208	12.101 ± 0.05	13.8 ± 0.3	114 ± 2
*Computed average values from all 6 measurements				

Table 2.3.2 Comparison of certified and measured concentrations of multiple elements in standard reference material SRM 1643f

Reference Material	Element of Interest	Certified Concentration (µg/L)	Measured Concentration (µg/L)*	Accuracy (%)
	Be 9	13.67 ± 0.12	12.4 ± 0.9	91 ± 6
	Al 27	133.8 ± 1.2	132 ± 12	98 ± 9
	V 51	36.07 ± 0.28	35.3 ± 0.9	98 ± 3
	Cr 52	18.5 ± 0.1	18.2 ± 0.4	99 ± 2
	Mn 55	37.14 ± 0.6	36 ± 1	97 ± 3
	Co 59	25.3 ± 0.17	24.9 ± 0.6	98 ± 2
	Ni 60	59.8 ± 1.4	54.6 ± 0.9	91 ± 2
	Cu 63	21.66 ± 0.71	20.8 ± 0.6	96 ± 3
1643f	Zn 66	74.4 ± 1.7	72 ± 3	97 ± 4
	As 75	57.42 ± 0.38	53.8 ± 1.5	94 ± 3
	Ag 107	0.9703 ± 0.0055	0.8 ± 0.2	81 ± 24
	Cd 111	5.89 ± 0.13	5.51 ± 0.08	94 ± 1
	Sb 121	55.45 ± 0.4	53.7 ± 1.2	97 ± 2
	Tl 205	6.892 ± 0.035	6.5 ± 0.1	94 ± 2
	Pb 208	18.448 ± 0.084	17.6 ± 0.3	96 ± 2
*Computed average values from all 5 measurements				

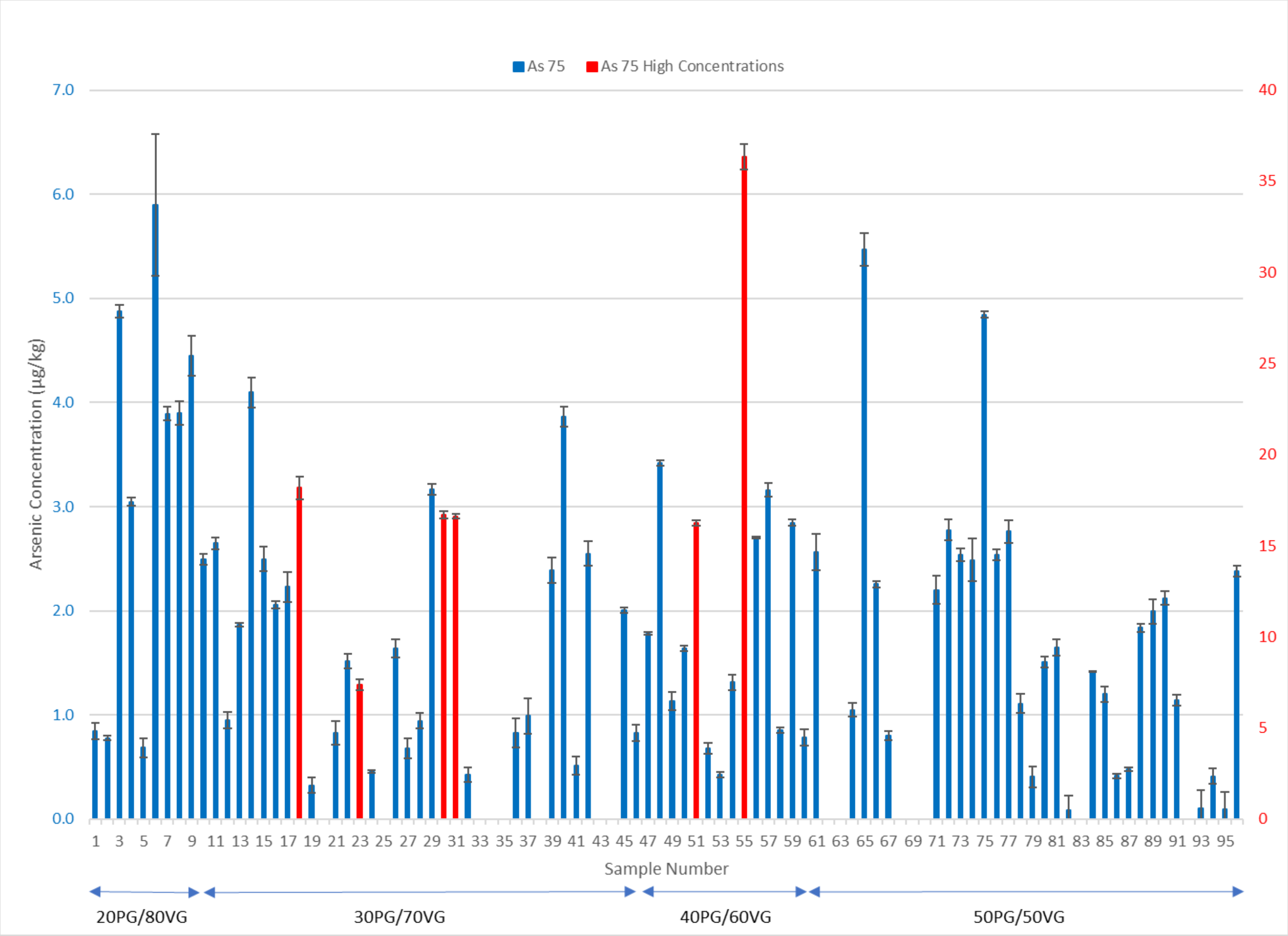

Figure 2.3.3 Total arsenic concentrations (µg/kg) found in 96 e-liquid samples. The blue colour represents arsenic concentrations (µg/kg) less than 7 µg/kg (scale on the left-hand side vertical axis), while the red colour represents arsenic concentrations greater than 7 µg/kg (scale on the right-hand side vertical axis)

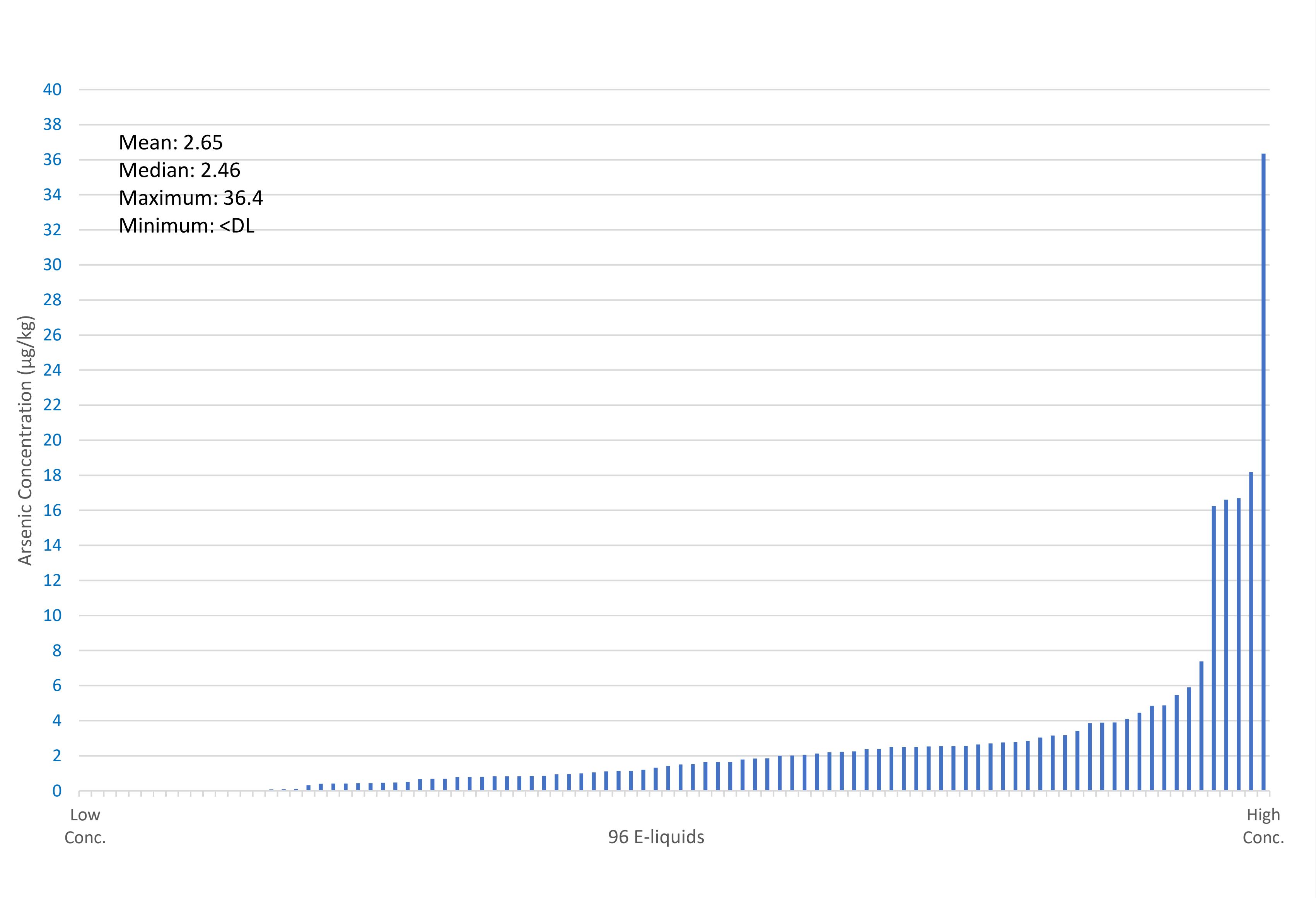

Figure 2.3.4 Total arsenic concentrations (µg/kg) arranged from low to high in e-liquids (n = 96)

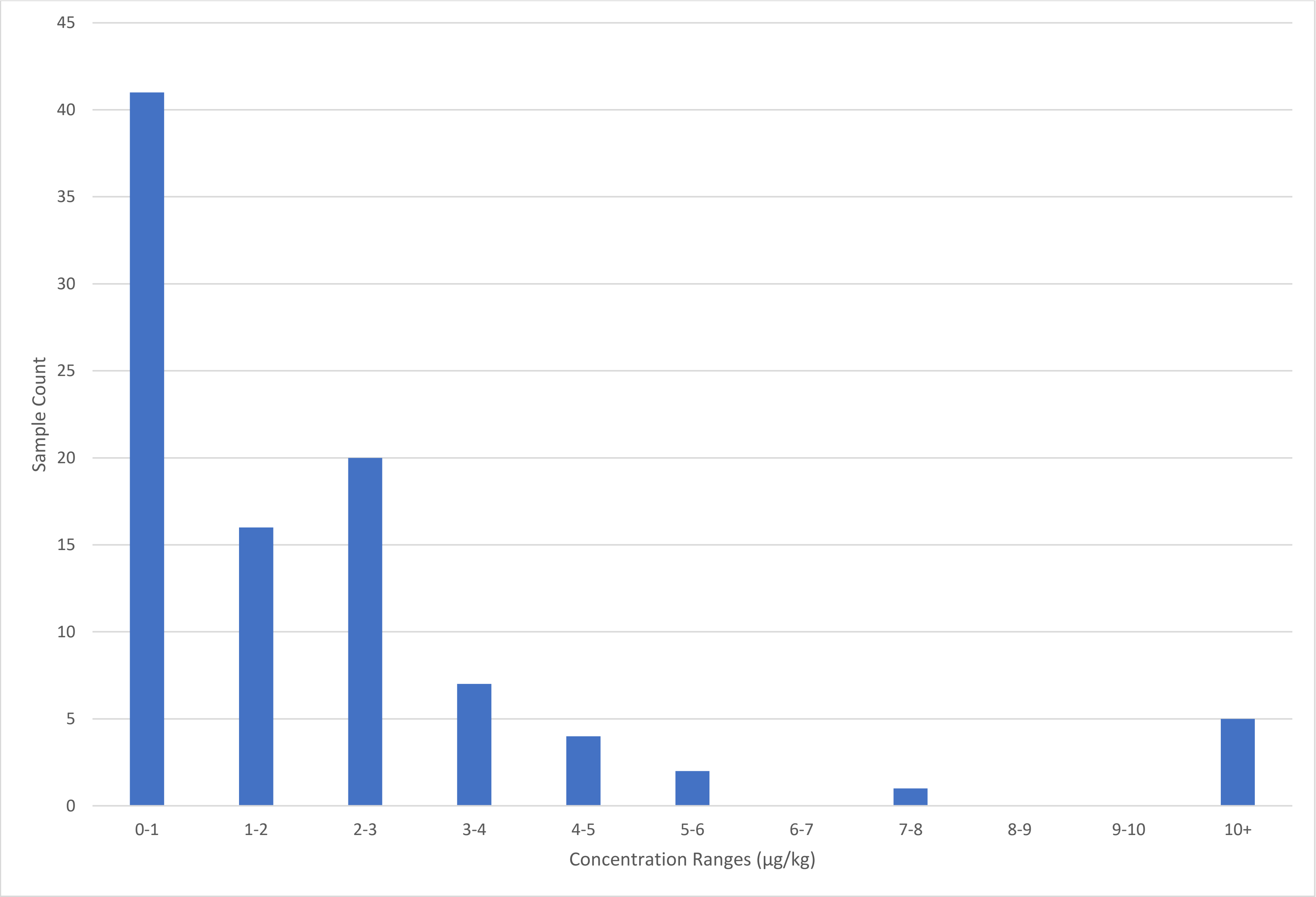

Figure 2.3.5 Distribution of total arsenic concentrations (µg/kg) in e-liquids (n = 96)

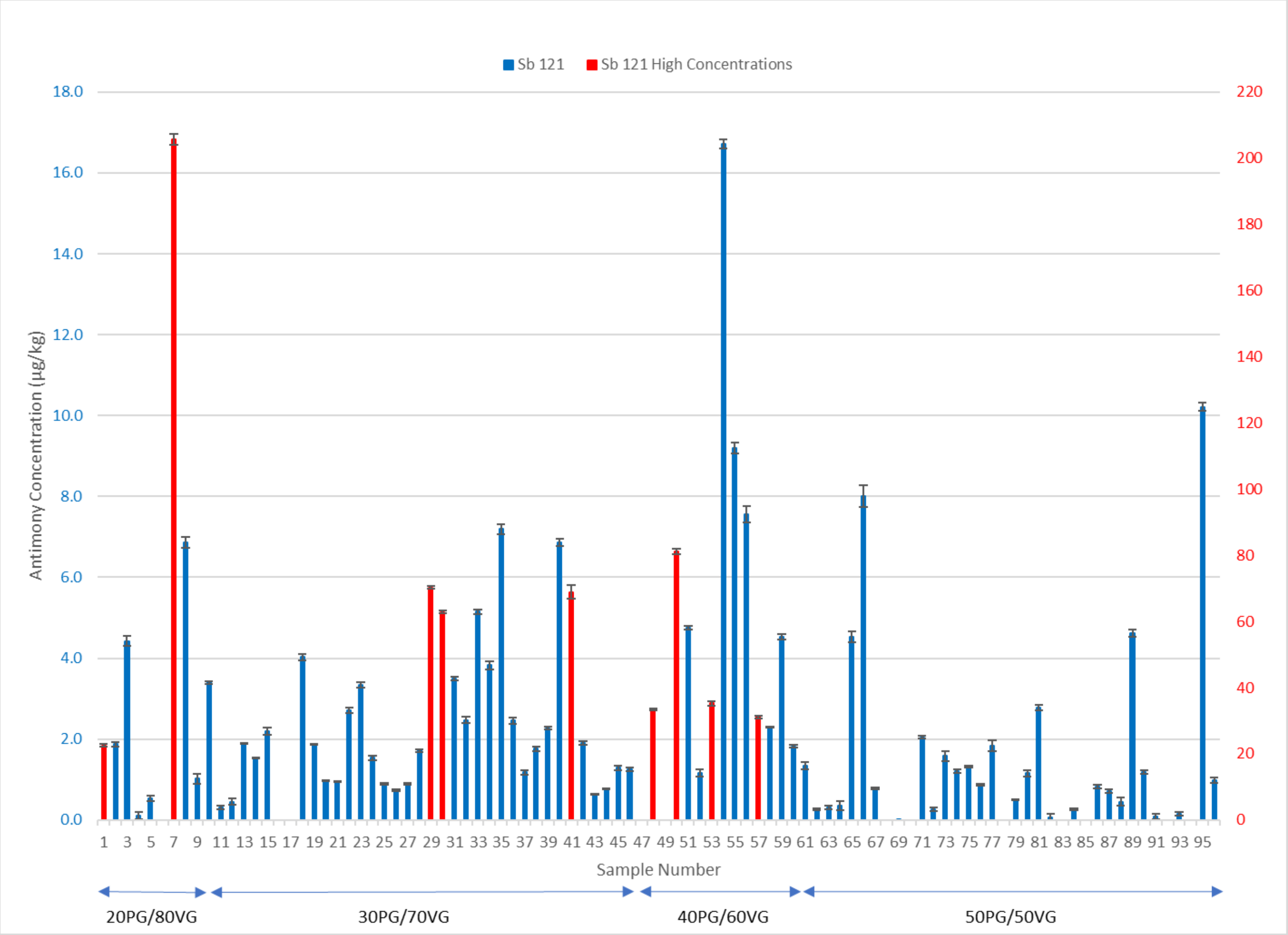

Figure 2.3.6 Total antimony concentrations (µg/kg) found in 96 e-liquid samples. The blue colour represents antimony concentrations (µg/kg) less than 18 µg/kg (scale on the left-hand side vertical axis), while the red colour represents antimony concentrations greater than 18 µg/kg (scale on the right-hand side vertical axis)

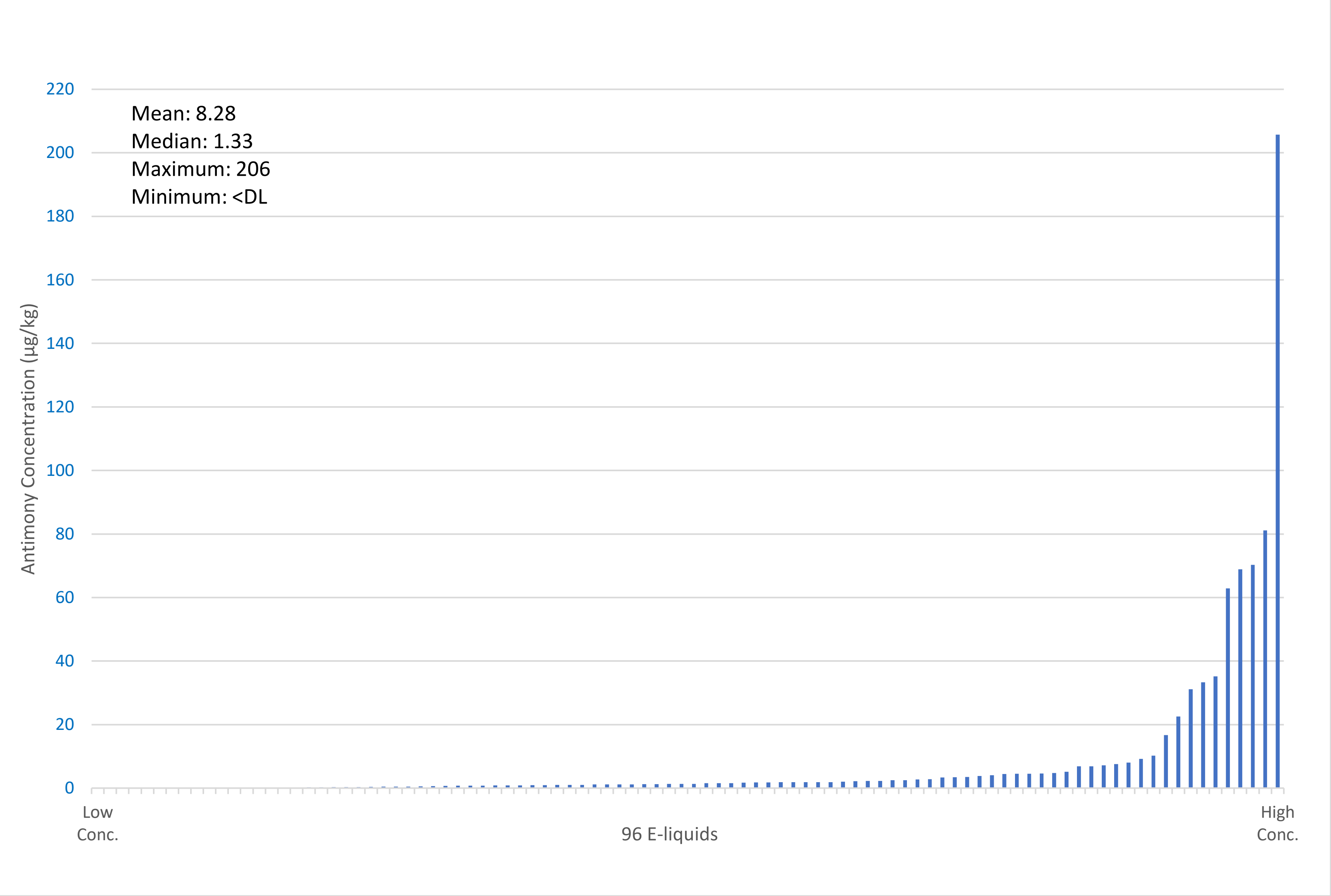

Figure 2.3.7 Total antimony concentrations (µg/kg) arranged from low to high in e-liquids (n = 96)

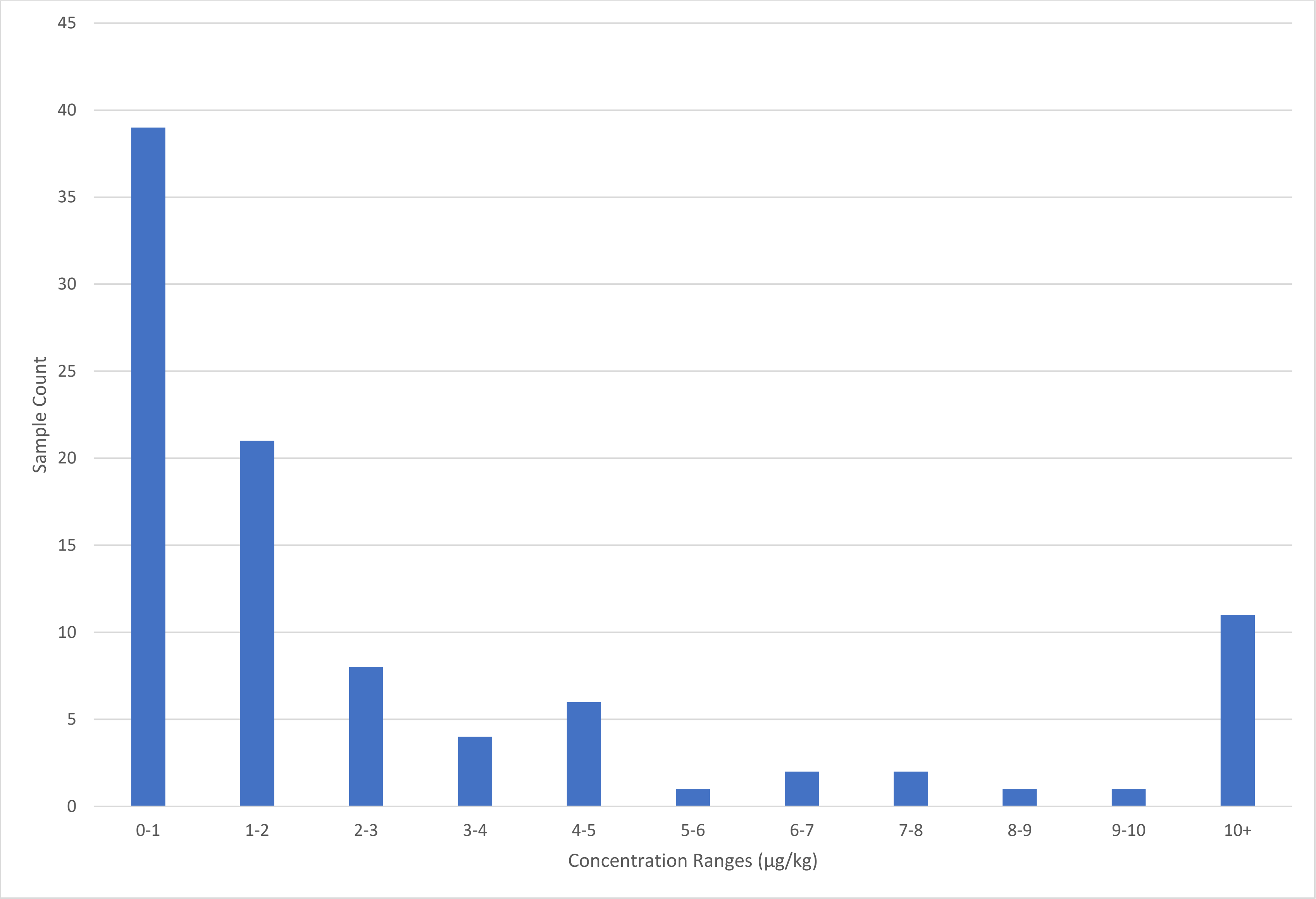

Figure 2.3.8 Distribution of antimony concentrations (µg/kg) in e-liquids (n = 96)

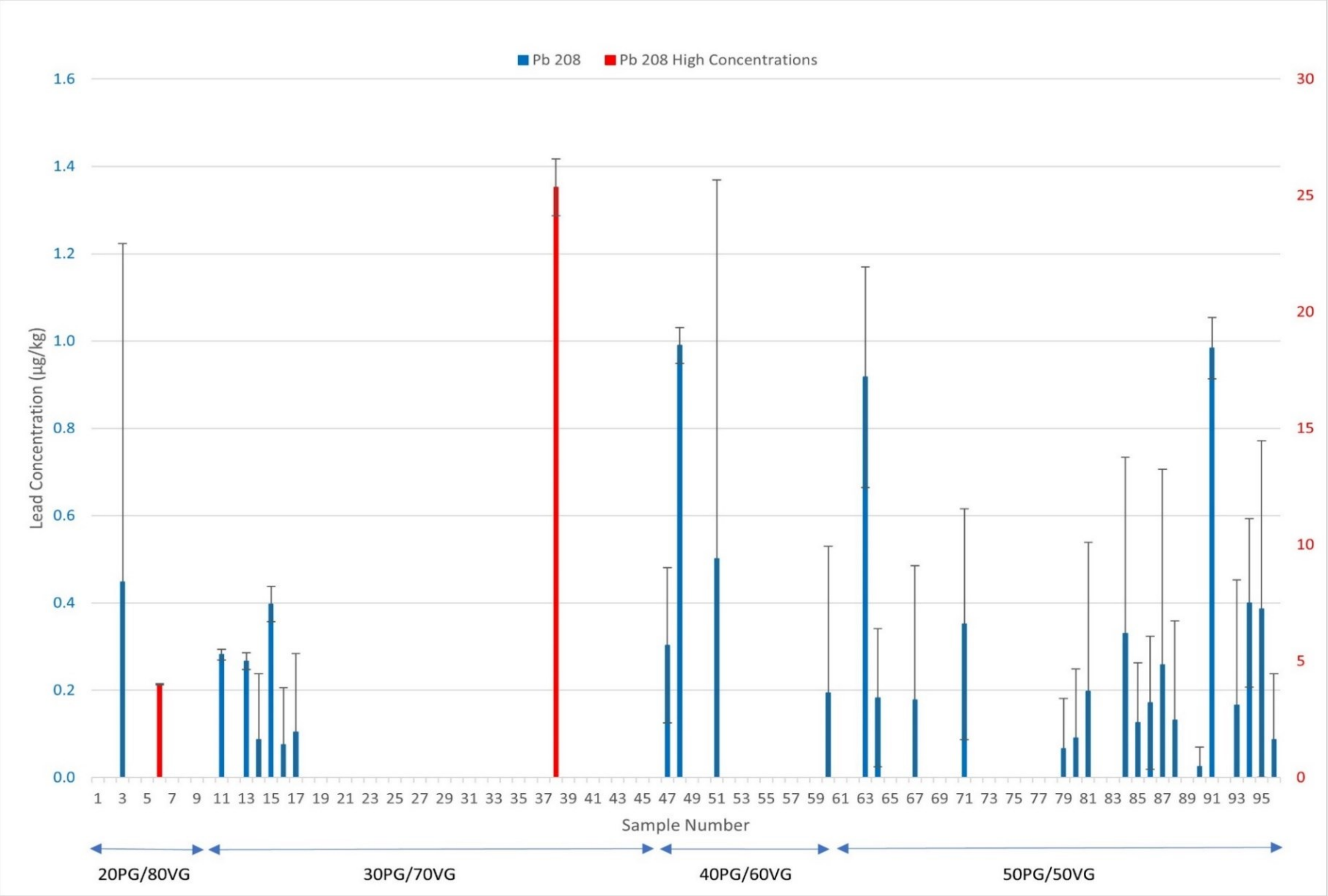

Figure 2.3.9 Total lead concentrations (µg/kg) found in 96 e-liquid samples. The blue colour represents lead concentrations (µg/kg) less than 1 µg/kg (scale on the left-hand side vertical axis), while the red colour represents lead concentrations greater than 1 µg/kg (scale on the right-hand side vertical axis)

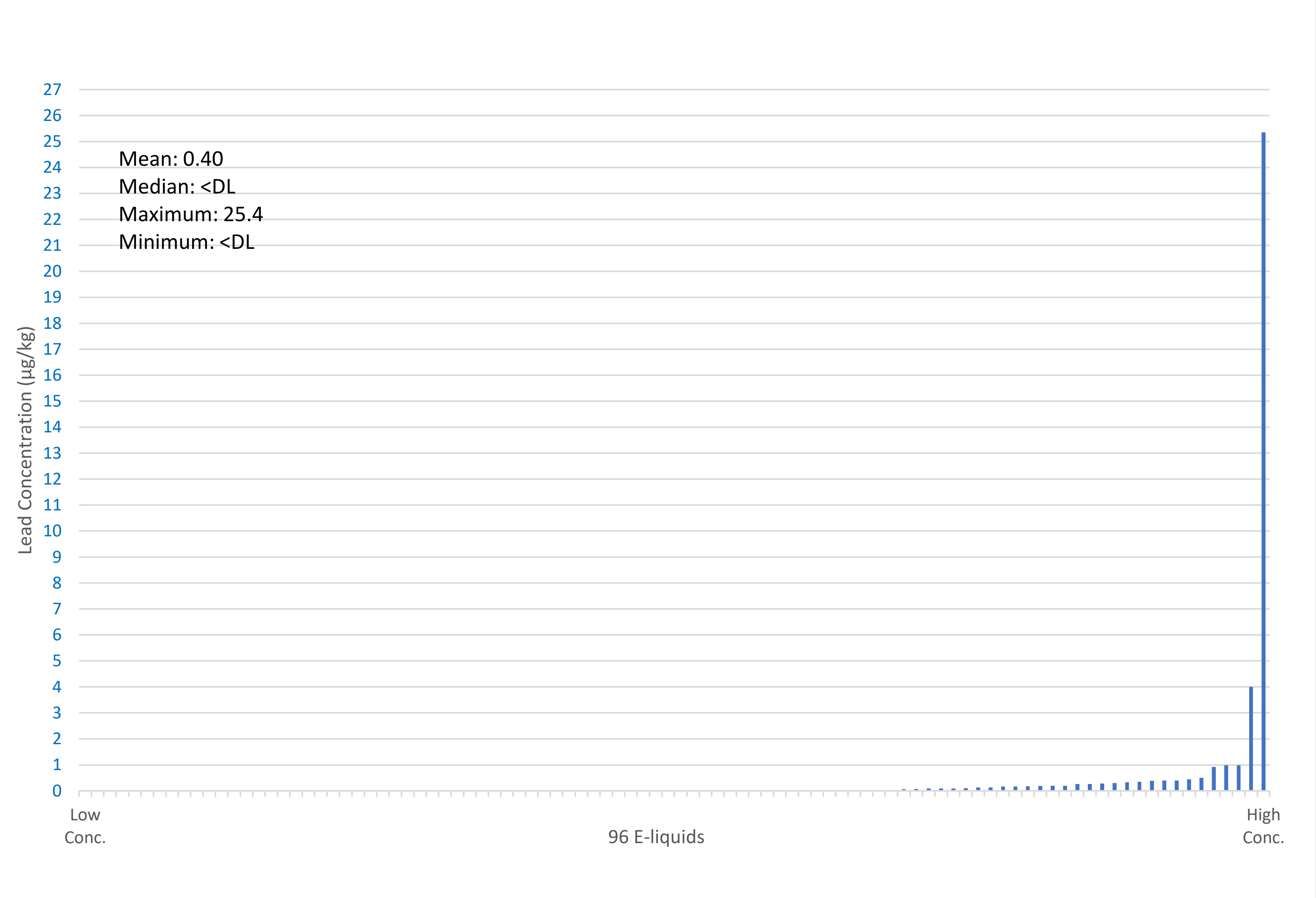

Figure 2.3.10 Total lead concentrations (µg/kg) arranged from low to high in e-liquids (n = 96)

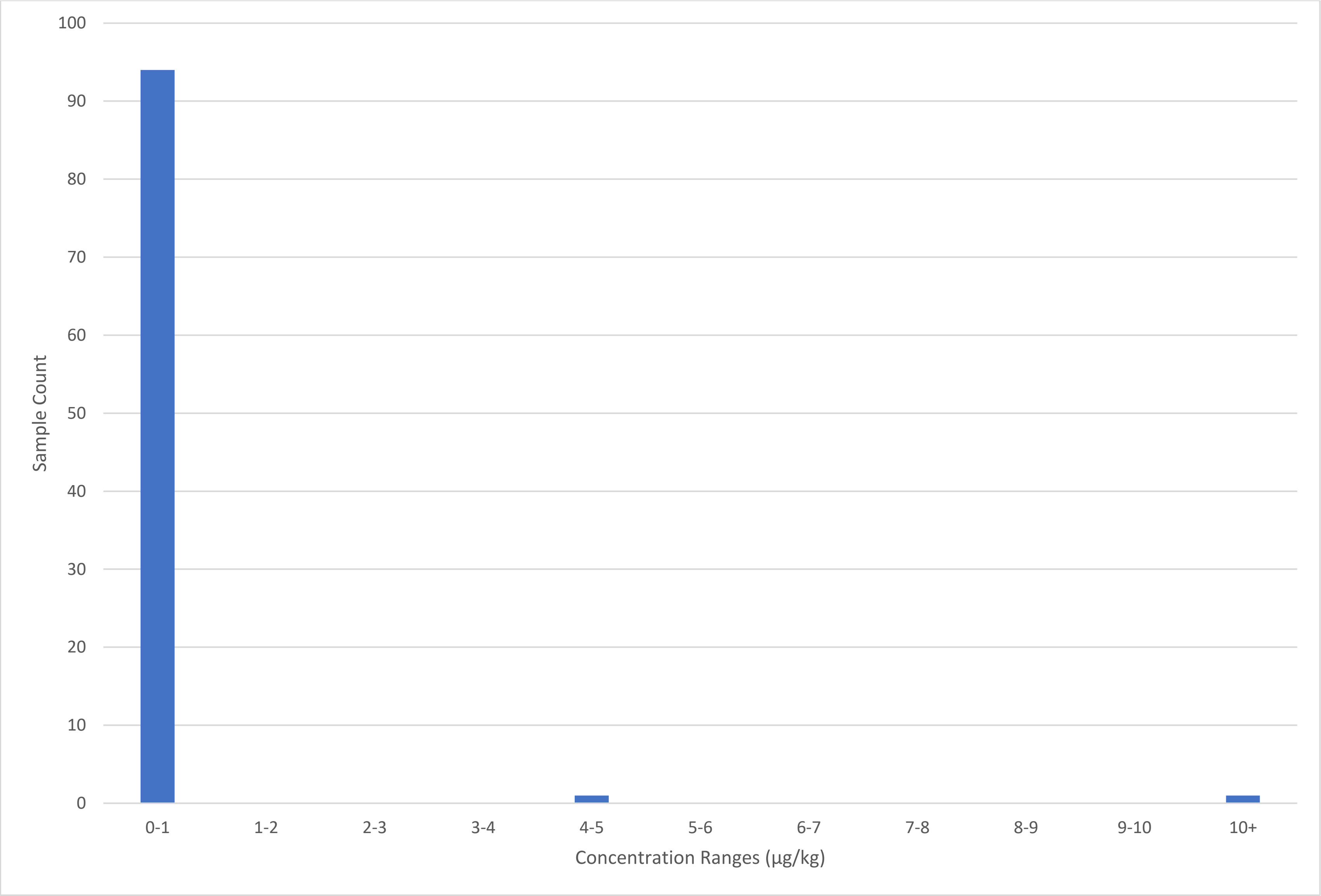

Figure 2.3.11 Distribution of lead concentrations (µg/kg) in e-liquids (n = 96)

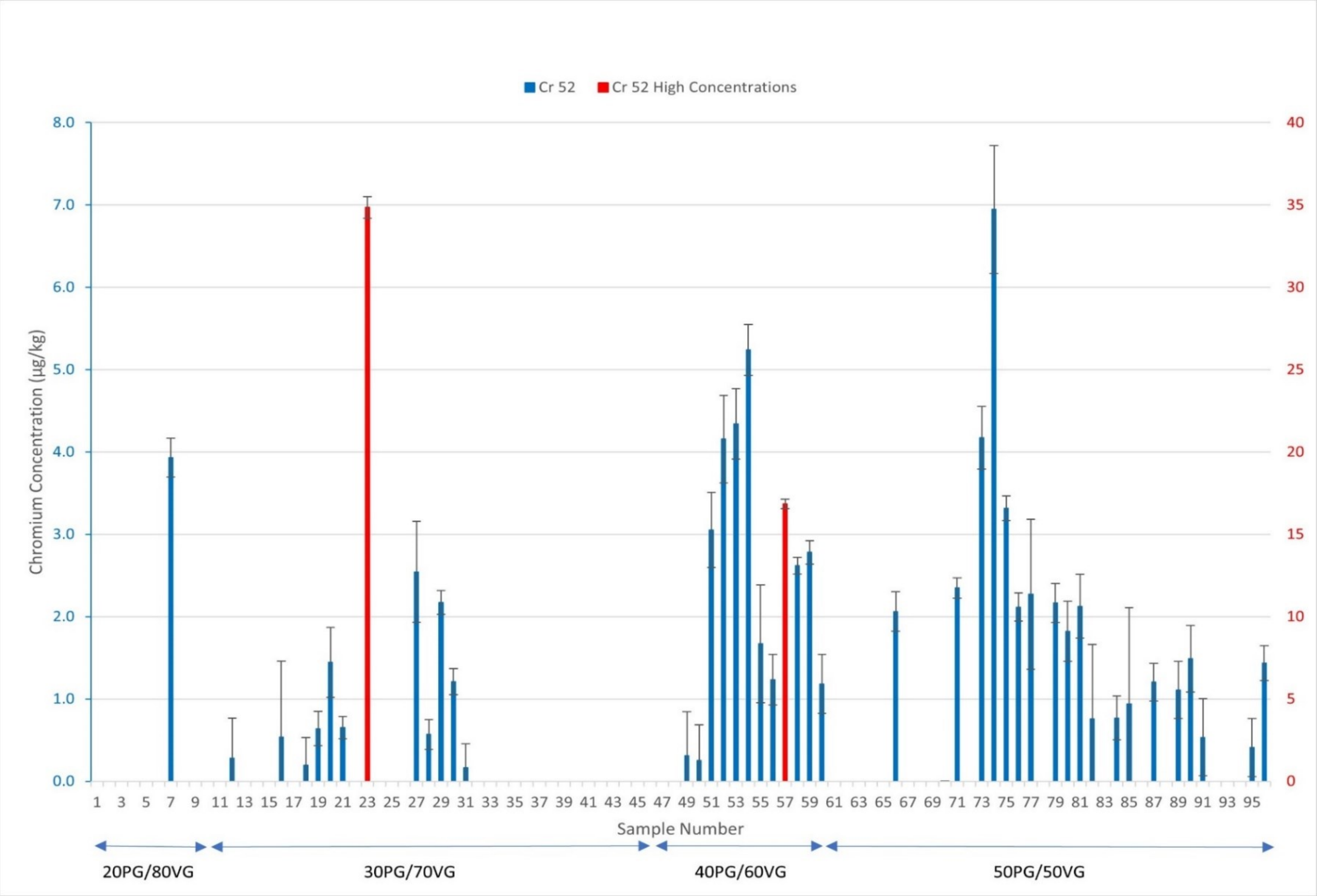

Figure 2.3.12 Total chromium concentrations (µg/kg) found in 96 e-liquid samples. The blue colour represents chromium concentrations (µg/kg) less than 7 µg/kg (scale on the left-hand side vertical axis), while the red colour represents chromium concentrations greater than 7 µg/kg (scale on the right-hand side vertical axis)

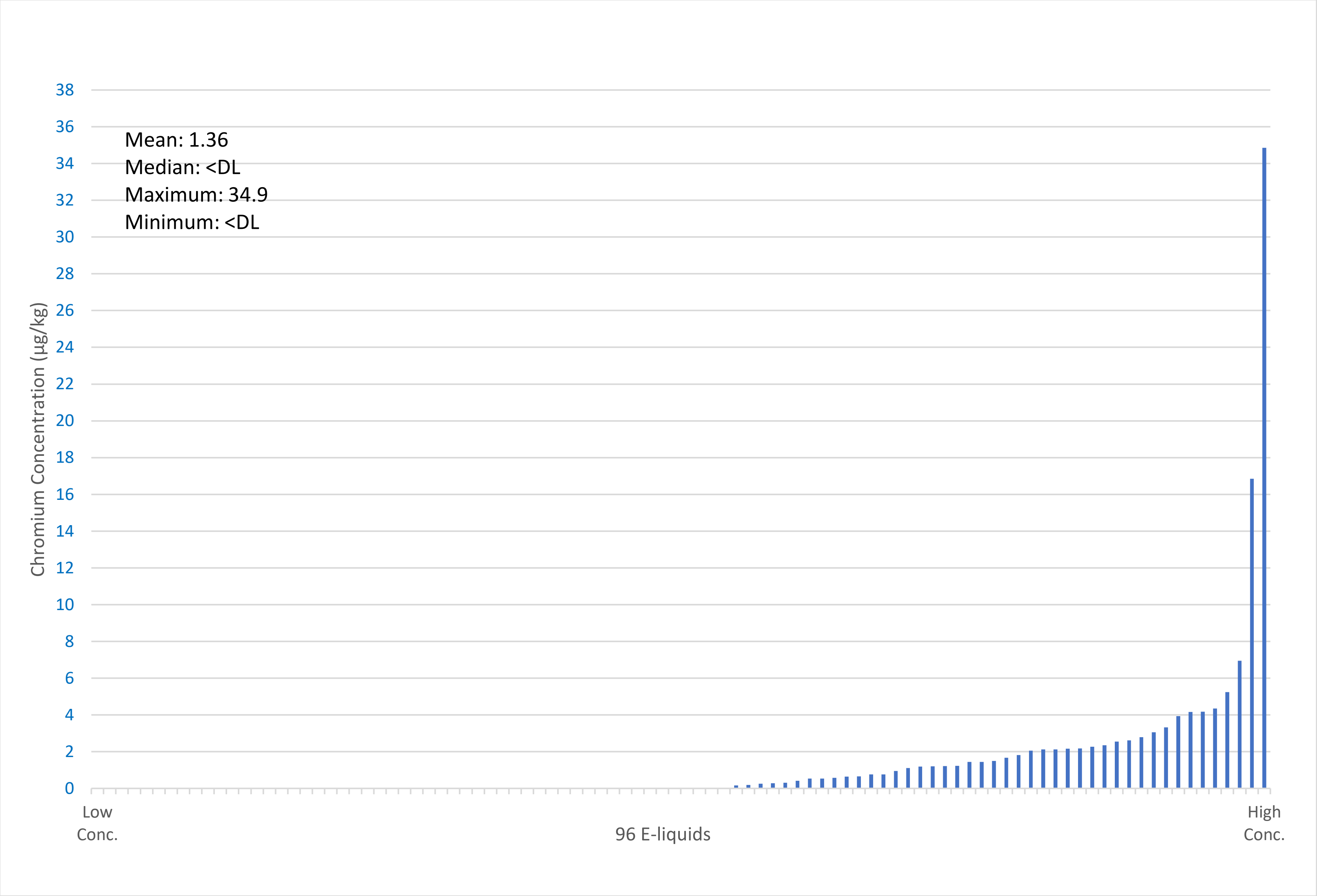

Figure 2.3.13 Total chromium concentrations (µg/kg) arranged from low to high in e-liquids (n = 96)

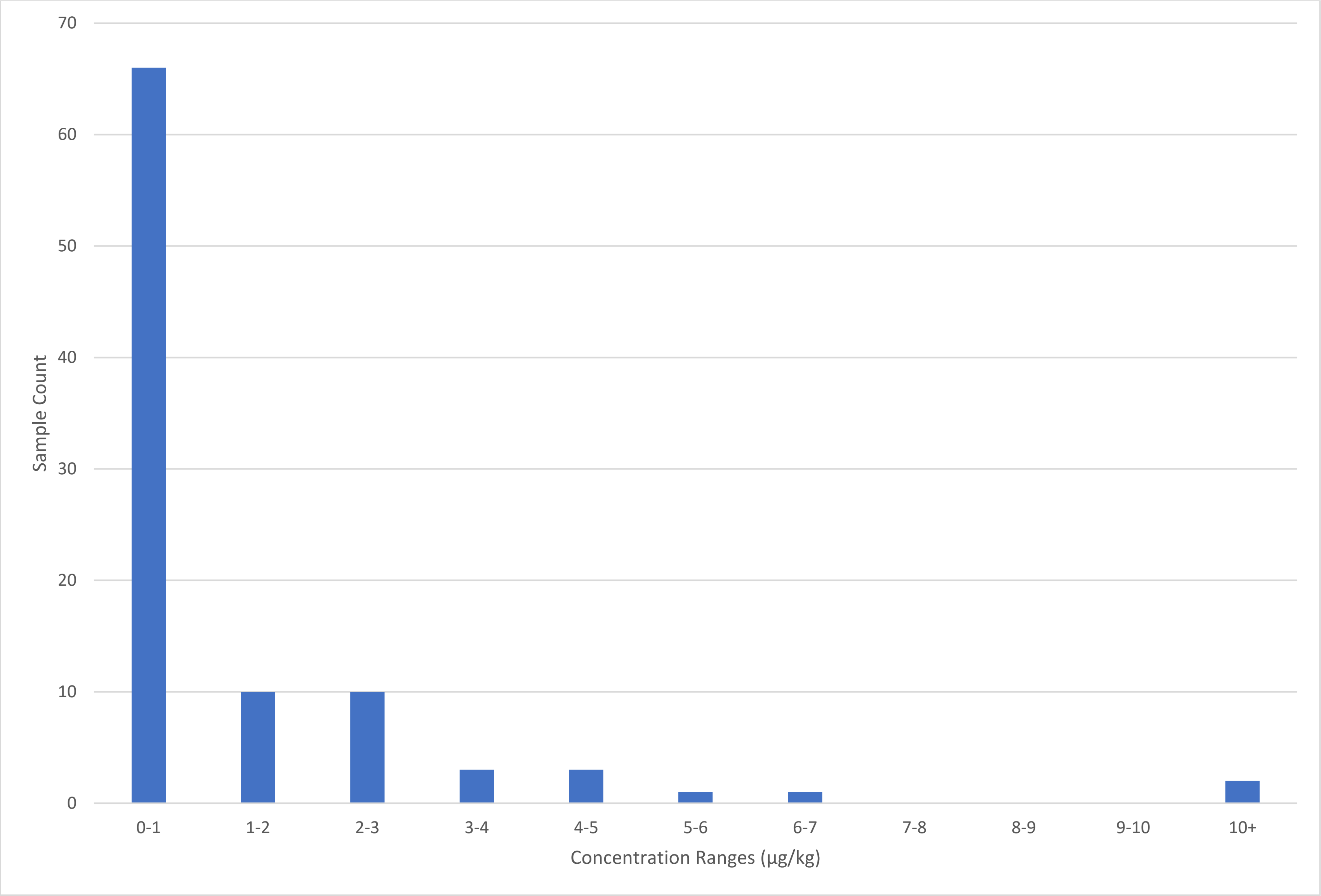

Figure 2.3.14 Distribution of chromium concentrations (µg/kg) in e-liquids (n = 96)

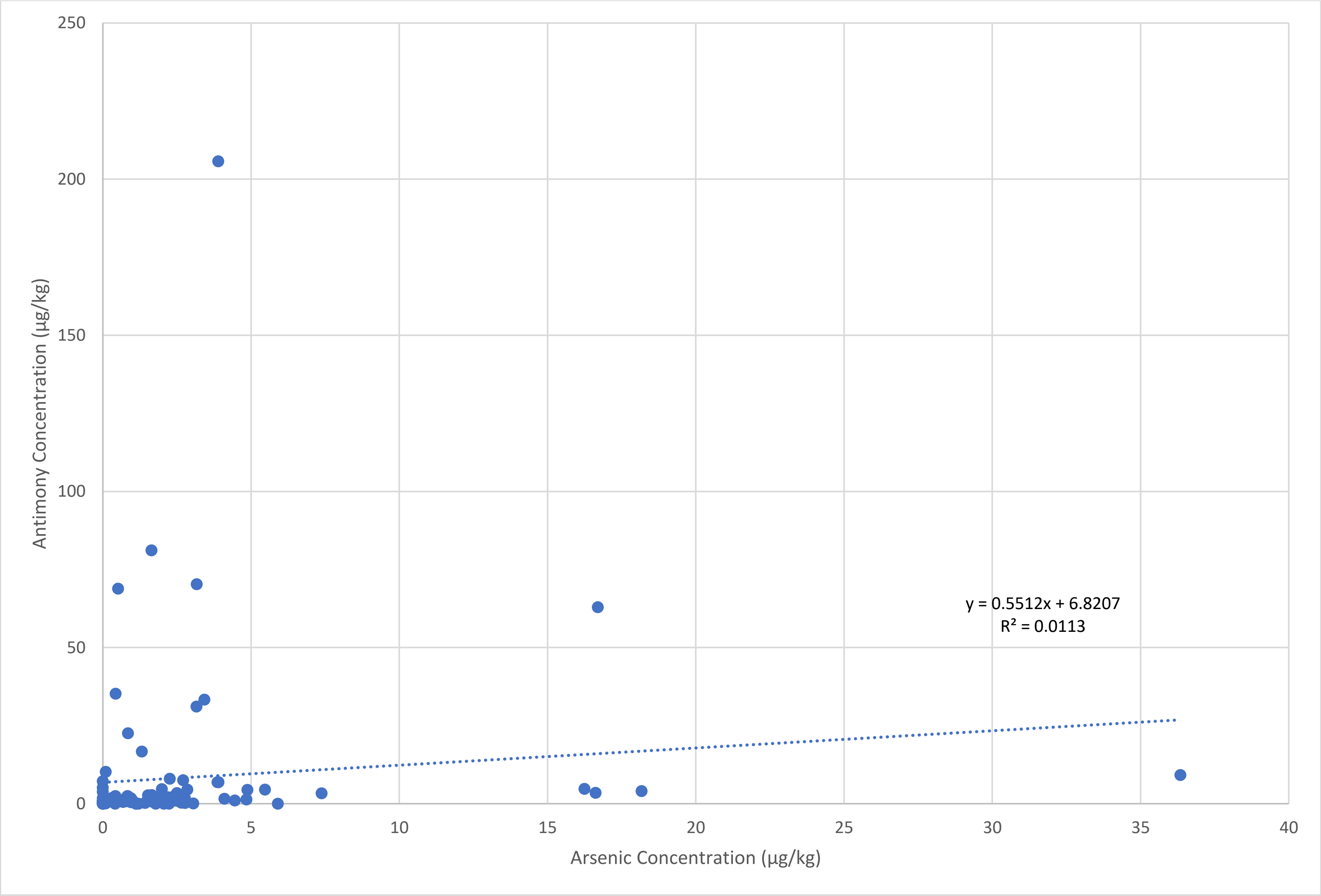

Figure 2.3.15 Correlation between antimony (µg/kg) and arsenic (µg/kg) in e-liquids

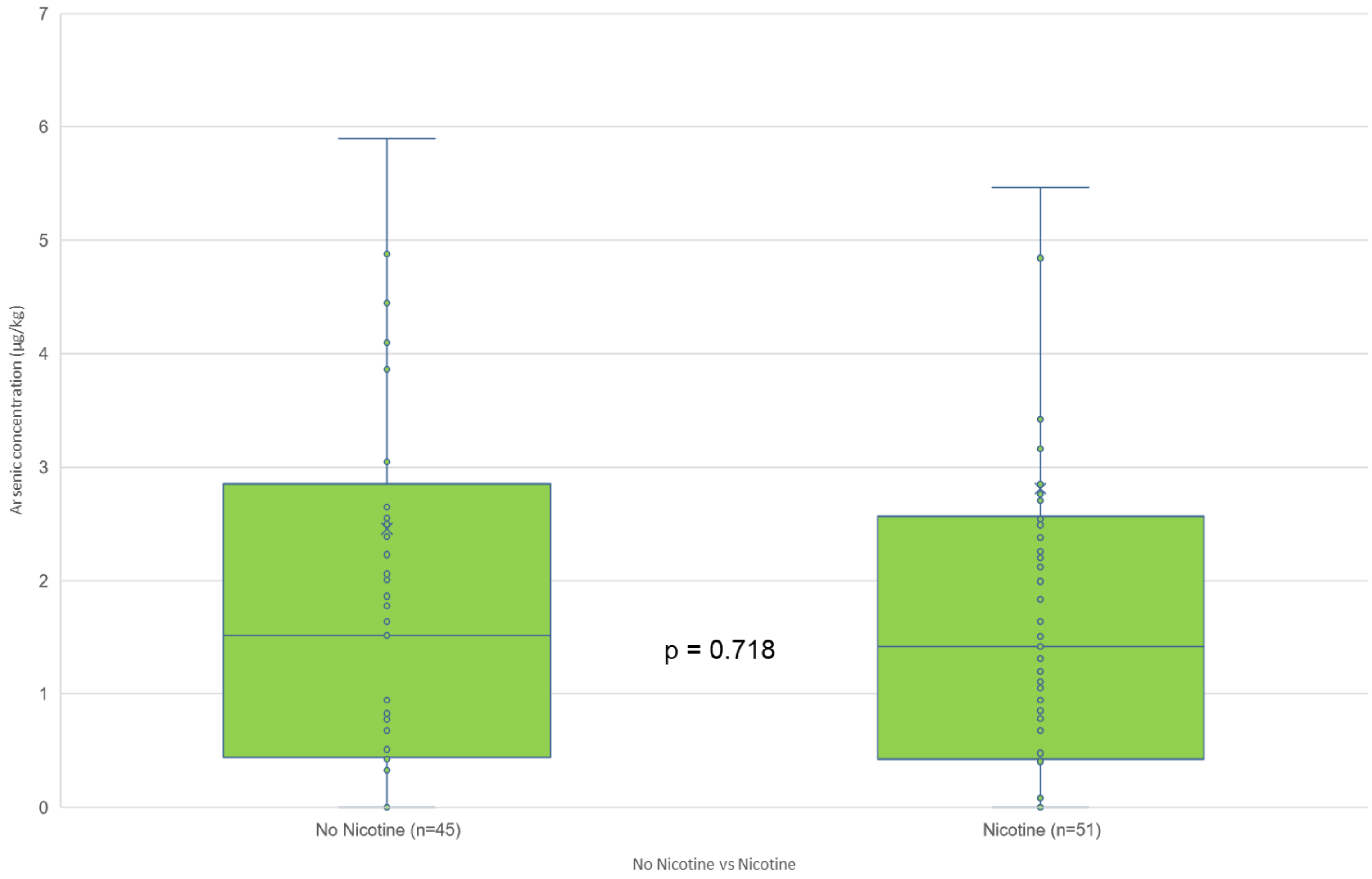

Figure 2.3.16 Comparison of e-liquid samples with and without nicotine, regarding total arsenic concentrations (µg/kg) found in 96 e-liquid samples. Outliers omitted from view *p = 0.719*

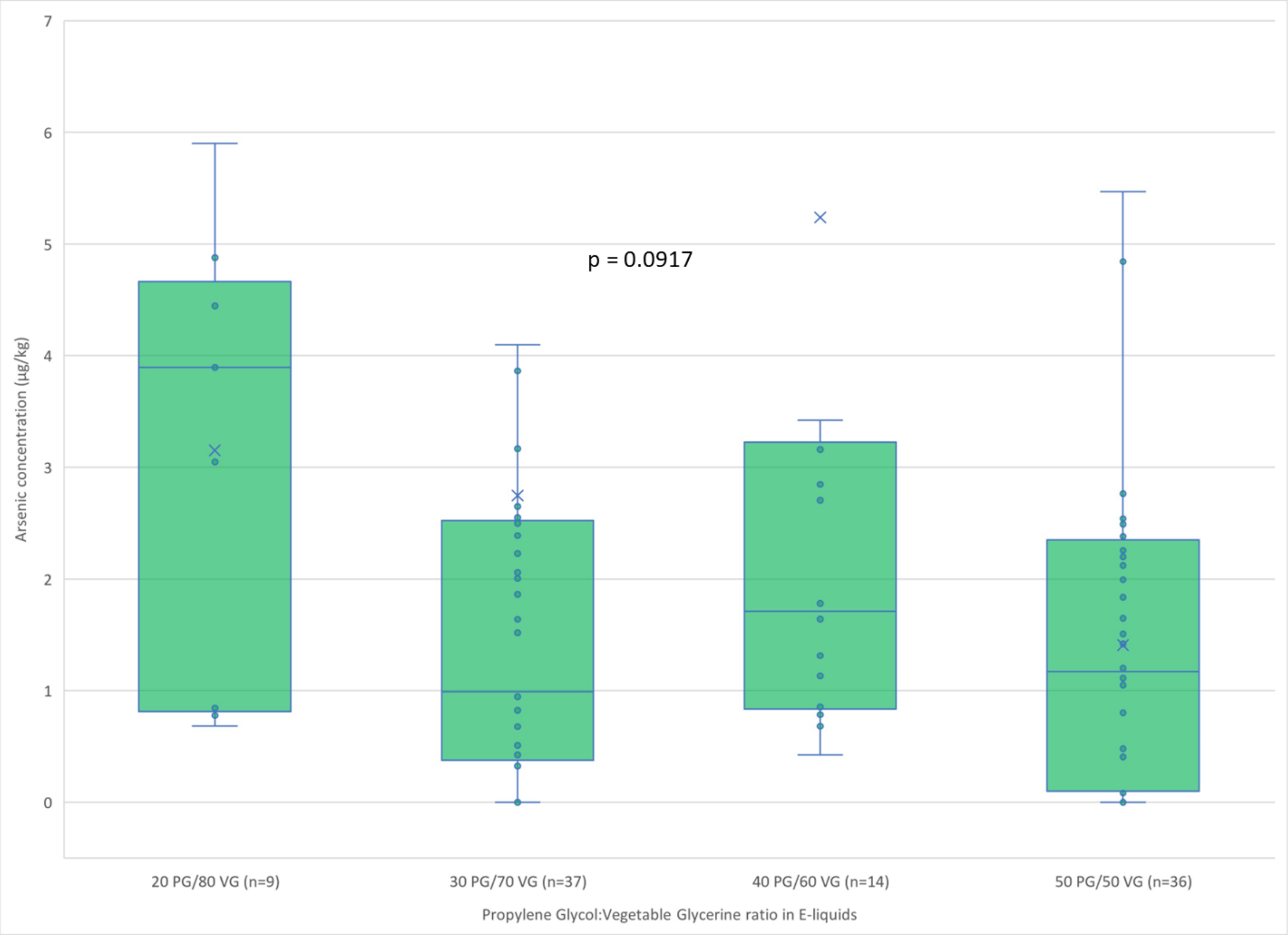

Figure 2.3.17 Comparison of total arsenic (µg/kg) in e-liquid samples between different PG:VG ratio of e-liquid base. Outliers omitted from view *p = 0.0917*

Table 2.3.3 Method Detection Limits (MDL) for all 15 elements investigated at various PG/VG ratios corresponding to the PG/VG ratios in the e-liquid samples

	20 PG/80 VG (µg/L)	**30 PG/70 VG (µg/L)**	**40 PG/60 VG (µg/L)**	**50 PG/50 VG (µg/L)**
Be 9	0.673	0.593	0.750	0.855
Al 27	2.664	1.600	3.454	2.326
V 51	0.010	0.010	0.008	0.005
Cr 52	0.033	0.103	0.035	0.029
Mn 55	0.020	0.021	0.011	0.012
Ni 58	0.013	0.019	0.012	0.008
Co 59	0.076	0.254	0.025	0.015
Ni 60	0.033	0.023	0.018	0.023
Cu 63	0.067	0.248	0.059	0.062
Zn 64	1.298	1.070	1.704	1.490
Zn 66	1.365	1.050	1.636	1.560
As 75	0.015	0.011	0.015	0.015
Ag 107	0.028	0.015	0.010	0.016
Cd 111	0.019	0.011	0.016	0.004
Cd 114	0.012	0.008	0.007	0.004
Sb 121	0.008	0.010	0.007	0.006
Tl 205	0.006	0.005	0.003	0.004
Pb 208	0.038	0.014	0.008	0.015

Table 2.3.4 Summary of e-liquid samples made with 20/80 PG/VG

Element	Total number of e-liquid samples analyzed	Samples with detectable concentrations (>MDL)	Percentage of samples having detectable concentrations (%)	Average concentration (µg/kg)	Median concentration (µg/kg)	Method Detection Limits (µg/L)	Maximum concentration (µg/kg)
Be 9	9	0	0	<DL	<DL	0.673	<DL
Al 27	9	2	22	9.6	<DL	2.664	67
V 51	9	6	67	0.48	0.54	0.010	1.32
Cr 52	9	1	11	0.4	<DL	0.033	3.9
Mn 55	9	0	0	<DL	<DL	0.020	<DL
Ni 58	9	3	33	0.3	<DL	0.013	1.6
Co 59	9	1	11	0.1	<DL	0.076	0.8
Ni 60	9	3	33	0.34	<DL	0.033	2.03
Cu 63	9	5	56	1.0	0.7	0.067	3.5
Zn 64	9	6	67	50	39	1.298	108
Zn 66	9	6	67	52	41	1.365	113
As 75	9	9	100	3.15	3.89	0.015	5.90
Ag 107	9	0	0	<DL	<DL	0.028	<DL
Cd 111	9	0	0	<DL	<DL	0.019	<DL
Cd 114	9	1	11	0.06	<DL	0.012	0.5
Sb 121	9	8	89	27.0	1.9	0.008	206
Tl 205	9	0	0	<DL	<DL	0.006	<DL
Pb 208	9	2	22	0.5	<DL	0.038	4

Table 2.3.5 Summary of e-liquid samples made with 30/70 PG/VG

Element	Total number of e-liquid samples analyzed	Samples with detectable concentrations (>MDL)	Percentage of samples having detectable concentrations (%)	Average concentration (µg/kg)	Median concentration (µg/kg)	Method Detection Limits (µg/L)	Maximum concentration (µg/kg)
Be 9	37	0	0	<DL	<DL	0.593	<DL
Al 27	37	2	5	2	<DL	1.600	54
V 51	37	22	60	0.80	0.18	0.010	17.53
Cr 52	37	12	32	1.2	<DL	0.103	34.9
Mn 55	37	24	65	3.9	0.3	0.021	49.8
Ni 58	37	8	22	1.7	<DL	0.019	48.8
Co 59	37	8	22	0.3	<DL	0.254	5.0
Ni 60	37	21	57	2.5	0.2	0.023	59.3
Cu 63	37	10	27	5.5	<DL	0.248	73.7
Zn 64	37	26	70	27.6	9.0	1.070	333
Zn 66	37	28	76	29.0	9.5	1.050	345
As 75	37	29	78	2.75	0.99	0.011	18.17
Ag 107	37	1	3	<DL	<DL	0.015	0.1
Cd 111	37	12	32	<DL	<DL	0.011	0.1
Cd 114	37	7	19	0.01	<DL	0.008	0.2
Sb 121	37	35	95	7.41	1.88	0.010	70.3
Tl 205	37	7	19	0.0_1	<DL	0.005	0.2
Pb 208	37	7	19	0.7	<DL	0.014	25.4

Table 2.3.6 Summary of e-liquid samples made with 40/60 PG/VG

Element	Total number of e-liquid samples analyzed	Samples with detectable concentrations (>MDL)	Percentage of samples having detectable concentrations (%)	Average concentration (µg/kg)	Median concentration (µg/kg)	Method Detection Limits (µg/L)	Maximum concentration (µg/kg)
Be 9	14	0	0	<DL	<DL	0.750	<DL
Al 27	14	2	14	4	<DL	3.454	31
V 51	14	10	71	0.42	0.25	0.008	1.24
Cr 52	14	12	86	3.1	2.1	0.035	16.9
Mn 55	14	12	86	3.8	2.1	0.011	18.1
Ni 58	14	13	93	1.7	1.3	0.012	5.4
Co 59	14	3	21	0.1	<DL	0.025	1.2
Ni 60	14	12	86	2.0	1.8	0.018	5.8
Cu 63	14	13	93	26	13	0.059	92
Zn 64	14	9	64	39	21	1.704	257
Zn 66	14	9	64	41	22	1.636	268
As 75	14	14	100	5.24	1.71	0.015	36.35
Ag 107	14	0	0	<DL	<DL	0.010	<DL
Cd 111	14	0	0	<DL	<DL	0.016	<DL
Cd 114	14	1	7	0.0_2	<DL	0.007	0.3
Sb 121	14	12	86	16.4	6.2	0.007	81.2
Tl 205	14	0	0	<DL	<DL	0.003	<DL
Pb 208	14	4	29	0.1	<DL	0.008	1

Table 2.3.7 Summary of e-liquid samples made with 50/50 PG/VG

Element	Total number of e-liquid samples analyzed	Samples with detectable concentrations (>MDL)	Percentage of samples having detectable concentrations (%)	Average concentration (µg/kg)	Median concentration (µg/kg)	Method Detection Limits (µg/L)	Maximum concentration (µg/kg)
Be 9	36	0	0	<DL	<DL	0.855	<DL
Al 27	36	8	22	10	<DL	2.326	73
V 51	36	25	69	0.80	0.13	0.005	9.36
Cr 52	36	20	56	1.1	0.5	0.029	7.0
Mn 55	36	10	28	2.9	<DL	0.012	60.0
Ni 58	36	25	69	1.1	0.4	0.008	11.1
Co 59	36	7	19	0.1	<DL	0.015	0.8
Ni 60	36	20	56	1.2	0.1	0.023	14.2
Cu 63	36	34	94	13	5	0.062	68
Zn 64	36	20	56	24	12	1.490	96
Zn 66	36	20	56	25	12	1.560	101
As 75	36	29	81	1.41	1.17	0.015	5.47
Ag 107	36	0	0	<DL	<DL	0.016	<DL
Cd 111	36	2	6	0.0_1	<DL	0.004	0.2
Cd 114	36	4	11	0.0_1	<DL	0.004	0.2
Sb 121	36	29	81	1.35	0.61	0.006	10.21
Tl 205	36	0	0	<DL	<DL	0.004	<DL
Pb 208	36	18	50	0.1	0.0_1	0.015	1.0

Table 2.3.8 Summary of all 96 e-liquid samples

Element	**Total number of e-liquid samples analyzed**	**Samples with detectable concentrations (>MDL)**	**Percentage of samples having detectable concentrations (%)**	**Maximum concentration (µg/kg)**
Be 9	96	0	0	<DL
Al 27	96	14	15	73
V 51	96	63	66	17.53
Cr 52	96	45	47	34.9
Mn 55	96	46	48	60.0
Ni 58	96	49	51	48.8
Co 59	96	19	20	5.0
Ni 60	96	56	58	59.3
Cu 63	96	62	65	92
Zn 64	96	61	64	333
Zn 66	96	63	66	345
As 75	96	81	84	36.35
Ag 107	96	1	1	0.1
Cd 111	96	14	15	0.2
Cd 114	96	13	14	0.5
Sb 121	96	84	88	206
Tl 205	96	7	7	0.2
Pb 208	96	31	32	25.4

2.4 Discussion

2.4.1 Calibration curves in the presence of matrix and SRM values

A series of calibration curves were prepared with each having a different e-liquid matrix solution added to them. The slopes of those calibration curves were plotted against the specific e-liquid matrix solution (PG/VG) that were added to them as shown in Figure 2.3.1. The trend shows that as the percentage of PG/VG increases, the slope of the calibration curves increases suggesting that sensitivity changes as the percentage of PG/VG changes which could be because PG is less dense than VG. This trend shown was observed for most of the 14 elements analyzed except for beryllium, aluminum, and silver. Based on this preliminary data, calibration curves and quality control samples were made in the presence of e-liquid matrix that corresponded to the percentage of PG/VG in the samples being analyzed that day. Calibration curves for all elements except for aluminum were very good, having R^2 values of 0.9975 or higher. A representative calibration curve for arsenic is shown in Figure 2.3.2.

To check the accuracy of the method, standard reference materials 1640a and 1643f were used and run for every analysis. For SRM 1640a, most values obtained were within ±10% of the certified values except for aluminum (+12%), cobalt (+13%), thallium (+11%), and lead (+14%). Elements such as arsenic, chromium, copper, nickel, and silver were all within ±5% of the certified value with arsenic being the closest at 102 ± 1% as shown in Table 2.3.1. The values obtained for SRM 1643f were mostly within ±5% of the certified values except for beryllium (-9%), nickel (-9%), arsenic (-6%), silver (-19%), cadmium (-6%), and thallium (-6%) as shown in Table 2.3.2. When the results of SRM 1643f were compared to SRM 1640a it was noted that values were below 100% whereas all the values for SRM 1640a were above 100%.

2.4.2 Concentration of arsenic and 14 other elements in samples

Table 2.3.8 summarizes the percentage of samples that had concentrations above the method detection limits of all 15 elements investigated for the 96 e-liquid samples analyzed. While each analyte’s detection percentage varied, it should be noted that, except for beryllium, silver, and thallium, all elements investigated were detected in at least 10% of the 96 samples analyzed. Arsenic and antimony were both detected in the majority of the e-liquids, having been detected in 84% and 88% of samples respectively. Having similar detection rates is consistent with previous literature on arsenic and antimony coexistence (Chang et al., 2022; Guo et al., 2022; Lehr et al., 2007; Wilson et al., 2010). The concentration of total antimony was plotted against the concentration of total arsenic to determine if there is a relationship. As shown in Figure 2.3.15, an R^2 value of 0.0113 suggests that antimony and arsenic do not appear to have a correlation with each other in e-liquids. Other elements of concern, such as cadmium, chromium, cobalt, and lead were only detected in 14%, 47%, 20%, and 32% of e-liquid samples respectively.

Although arsenic was detected in 81 out of the 96 e-liquids, most of these e-liquids had low concentrations, with 62 of the 81 samples having arsenic concentrations under 3 µg/kg. These low concentrations are consistent with previous work (Beauval et al., 2016; Olmedo et al., 2018, 2021; Song et al., 2018). Only eight e-liquids had arsenic concentration values above 5 µg/kg and of those eight, five of them had concerning amounts of arsenic present, with concentrations ranging between 16 µg/kg to 18 µg/kg for four of them and one sample having just above 36 µg/kg. Figure 2.3.3 graphically shows the various concentration of arsenic in all 96 e-liquid samples with small standard deviations as shown by the small error bars suggesting good sample reproducibility. Figure 2.3.4 and Figure 2.3.5 further demonstrate that most of the e-liquid samples had arsenic concentrations below 3 µg/kg.

Since nicotine is an optional addition in e-liquids, the presence of nicotine was investigated to determine if it was a major source of arsenic found in e-liquids. As shown in Figure 2.3.16, the box plot comparison, along with ANOVA analysis, yielded a p-value of 0.718 which is larger than the alpha value of 0.05 suggesting that there is no statistical difference in arsenic concentrations between e-liquids that have no nicotine and e-liquids that have nicotine.

As mentioned previously, e-liquids are made using a variety of PG/VG ratios. The concentration of arsenic between the four different PG/VG ratios found in the e-liquid samples analyzed was investigated as shown in Figure 2.3.17. From the ANOVA analysis, a p-value of 0.0917 was obtained suggesting that there is no statistical difference in the concentration of arsenic between the four PG/VG ratios analyzed.

Antimony was detected in 84 of the 96 e-liquid samples with 56 of them having concentrations below 3 µg/kg. Eleven e-liquids had concentrations above 10 µg/kg, with ten of them ranging between about 20 µg/kg to 81 µg/kg and one of them with a concentration of 205 µg/kg. When compared to literature, these results are higher overall (Beauval et al., 2016; Olmedo et al., 2018, 2021). These results are shown graphically in Figure 2.3.6 and had high precision, similar to arsenic, as shown by the small standard deviations. Figure 2.3.7 and Figure 2.3.8 also show that most of the e-liquids analyzed had antimony concentrations below 3 µg/kg.

Lead was only detected in 31 of the 96 e-liquid samples with 25 of them having concentrations below 0.5 µg/kg. When compared to literature these results are similar or lower than work done by Olmedo et al. (Olmedo et al., 2018, 2021). Only two e-liquid samples had higher concentrations of 4 µg/kg and 25 µg/kg. Lead had large standard deviation, and therefore low precision as shown in Figure 2.3.9. These large standard deviations were also observed for other elements such as aluminum, copper, and zinc and was attributed to matrix effects. Figure 2.3.10 and Figure 2.3.11 further demonstrate that most lead concentrations in the e-liquids analyzed were below 0.5 µg/kg.

Chromium was only detected in 45 of the 96 e-liquid samples with 35 of them having concentrations below 3 µg/kg. When compared to literature, these results are higher than work done by Olmedo et al. but lower than work done by Beauval et al. (Beauval et al., 2016; Olmedo et al., 2018). Only two e-liquid samples had high concentrations of chromium with corresponding values of 17 µg/kg and 35 µg/kg. The precision of chromium was moderate as shown by the small standard deviations in Figure 2.3.12. Figure 2.3.13 and Figure 2.3.14 further demonstrate that most of the e-liquids analyzed had chromium concentrations below 3 µg/kg.

Cobalt was only detected in 18 of the 96 e-liquid samples with 17 of them having concentrations below 3 µg/kg and only one sample having a concentration of 5.04 µg/kg. When compared to literature these results are higher (Beauval et al., 2016; Olmedo et al., 2021). Whereas cadmium was only detected in 14 of the 96 e-liquid samples with all of them having concentrations below 1 µg/kg.

2.4.3 Results of MDL determination

To mimic the e-liquid sample matrix, a premade mixture of PG and VG was added and diluted to a stock solution of 2% HNO_3 in deionized (18 megaohm) water. The final concentration of this PG/VG mixture was 5%. This stock solution was used to prepare samples (n = 9) spiked with 0.2, 0.3, 0.5, 1, 5, and 10 µg/L of the elements of interest. This procedure was repeated for all four different PG/VG ratios found in the e-liquid samples with the results shown in Table 2.3.3. For elements such as antimony, arsenic, thallium and vanadium the MDL's were not very different between the four PG/VG ratios while other elements such as aluminum and zinc had quite different MDL's.

2.5 Conclusions

Preliminary data investigating the effects of e-liquid matrix on the sensitivity suggested that the calibration curves needed to be made in the presence of e-liquid matrix. Specifically, the PG/VG ratio used to prepare the calibration curves should match the PG/VG ratio in the e-liquid samples. However, the MDL results show that for most elements, including arsenic, it does not have a large impact.

While nicotine is an optional additive to e-liquids, it does not have any statistically significant impact on the concentration of arsenic found in the 96 e-liquids analyzed. Additionally, the various PG/VG ratios that are used as carrier solvents in e-liquids also do not have statistically significant impacts on the concentration of arsenic found in e-liquids.

Arsenic along with 14 other contaminants were investigated in 96 e-liquid samples using ICP-MS. Concentrations of these elements varied between e-liquids, with most of them being in the low µg/kg or part per billion range with some exceptions. However, a few e-liquids did have concerning concentrations of arsenic, with concentrations as high as 36 µg/kg and antimony concentrations reached almost 206 µg/kg. This suggests that while overall exposure to most elements investigated from e-liquid consumption is low, it is not negligible, and can sometimes expose consumers to elevated concentrations of toxic elements.

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Chapter 3: Determination of Arsenic Species in Electronic Cigarette Liquid

3.1 Introduction

Arsenic exists in the environment in many different chemical forms, or chemical species. The toxicity of arsenic species varies greatly. For example, arsenite (As^{III}) one of the more toxic arsenic species has an LD_{50} value (median lethal dose) between 26-39 mg/kg in mice whereas arsenobetaine (AsB), considered virtually non-toxic, has an LD_{50} value >10000 mg/kg in mice (CONTAM, 2009). Therefore, quantification and identification of individual arsenic species are crucial for studies of arsenic speciation and health effects. Efficient separation techniques such as high performance liquid chromatography (HPLC) are commonly coupled with inductively coupled plasma mass spectrometry (ICP-MS) detection (Reid et al., 2020; Sadee et al., 2015). For arsenic speciation analysis, some common modes of chromatographic separation used include ion pair and anion exchange HPLC (Chen et al., 2010; Liu et al., 2015; Morita et al., 2007; Nan et al., 2018; Peng et al., 2014)

E-liquid analysis can be challenging because of the viscous nature of the e-liquid matrix, primarily caused by the propylene glycol (PG) and vegetable glycerine (VG) humectants used, which can make handling these samples difficult To overcome these challenges, large dilution factors are used, which can impact the detection of trace amounts of arsenic in e-liquid samples. Additionally, arsenic speciation analysis can be challenging when total arsenic concentration is low because individual arsenic species concentrations are even lower compared to total arsenic. While there have been several reports of arsenic found in e-liquids, the total arsenic concentrations reported have been quite low (Beauval et al., 2016; Olmedo et al., 2018, 2021). To date there have been very few studies on arsenic species in e-liquids (Liu et al., 2020).

In this chapter, determination of arsenic species in e-liquids was performed using ion pair HPLC with ICP-MS detection, and anion exchange HPLC with ICP-MS detection.

3.2 Materials and Methods

3.2.1 Reagents and Standards

Deionized water from a Milli-Q purification system (18.2 MΩ, Millipore, Molsheim, France) was used throughout these experiments. Standard 10 mg As/L stock solutions of arsenite (As^{III}), dimethylarsinic acid (DMA), monomethlyarsonic acid (MMA), and arsenate (As^{V}) were prepared by dissolving sodium arsenite (90%, Sigma-Aldrich, USA), cacodylic acid (98%, Sigma-Aldrich, USA), monosodium acid methane arsonate hydrate (99.5%, Chem Service, USA), and sodium arsenate dibasic heptahydrate (ACS reagent, 98%, Sigma-Aldrich, USA) in deionized water. Stock solutions were stored in a 4 °C fridge and arsenic standard solutions were prepared fresh each day from the stock solutions prior to analysis. Standard reference material 2669 level 2 (arsenic species in frozen human urine) was obtained from the National Institute of Standards and Technology (Gaithersburg, MD), and certified reference material CRM 18 (human urine) obtained from the National Institute for Environmental Studies (Ibaraki, Japan), were used as quality control measures to ensure accurate arsenic species determination in addition to evaluating calibration curve accuracy.

3.2.2 Sample Analysis

Eight e-liquid samples were selected from the group of 96 e-liquids described previously in chapter 2 and were chosen because they had total arsenic concentration values greater than 5 µg/kg and Table 3.2.1 shows the various characteristics of each sample.

Table 3.2.1 Characteristics of the eight e-liquid samples analyzed

Sample Number	PG/VG ratio	Bottle Size (mL)	Nicotine	Flavour	Total As Concentration (µg/kg)
1	25/75	100 mL	0	Blackberry	5.9 ± 0.7
2	30/70	30 mL	0	Banana smoothie and Strawberry	18.2 ± 0.6
3	30/70	60 mL	0	Chocolate Milk	7.4 ± 0.3
4	30/70	60 mL	3 mg/60 mL	Pineapple, Guava	16.7 ± 0.2
5	30/70	60 mL	3 mg/60 mL	Watermelon, Honeydew	16.6 ± 0.1
6	35/65	60 mL	0	Watermelon and Honeydew	16.2 ± 0.2
7	35/65	60 mL	0	Sweet Strawberry	36.4 ± 0.7
8	50/50	30 mL	25 mg/mL	Strawberry Melon	5.5 ± 0.2

After inverting each e-liquid sample bottle several times to ensure homogeneity, approximately 2.29 - 2.45 g of e-liquid was weighed into 15 mL polypropylene tubes and diluted 1:1 with deionized water to a total volume of 4 mL, before being vortexed. Each e-liquid sample was only weighed once, and after dilution was aliquoted into three HPLC vials before analysis.

Calibration solutions were prepared from the four arsenic standard solutions at the following concentrations (µg/L): 0.1, 0.2, 0.5, 1, 5, 10, and 20 µg/L. Calibration curves were evaluated using SRM 2669 for all four arsenic species, and CRM 18 for DMA, which were both prepared fresh with each analysis. Additionally, a 5 µg/L standard was used as a check standard to monitor instrument drift and was analyzed every 9 injections.

Determination of arsenic species in e-liquids was performed using an Agilent 1260 series II HPLC system (Agilent Technologies, Germany) coupled to a 7900 ICP-MS (Agilent Technologies, Santa Clara, U.S) for detection. ICP-MS conditions are shown in Table 3.2.1. Two methods of separation, ion pair and anion exchange, were investigated.

Table 3.2.2 Optimized 7900 ICP-MS parameters

Plasma	
RF Power	1550 W
RF matching	1.70 V
Sampling depth	8.0 mm
Nebulizer gas	1.09 L/min
Nebulizer pump	0.50 rps
S/C Temp	2 ^{o}C
Ion lenses	Optimized every run using a 1 µg/L Ce, Co, Li, Mg, Tl and Y in 2wt% HNO_3 tuning solution (Agilent Technologies, Santa Clara, U.S)
Collision cell	
He Flow	3.5 mL/min
OctP Bias	-18 V
OctP RF	200 V
Energy Discrimination	5 V
Data acquisition parameters	
Peak pattern	1 point
Integration time (per point)	0.5 s
Replicates	3

Ion pair chromatography separation was performed using an ODS-3 column (150 x 4.6 mm, 3 µm particle size, Phenomenex, USA) along with a guard column (Phenomenex, USA) and was kept at 50 °C throughout the analysis. Isocratic separation of arsenic species was done using a mobile phase comprised of 3 mM malonic acid (Reagent grade, Fisher Scientific, Japan), 5 mM tetrabutylammonium hydroxide (TBA) (40 wt. % in H_2O, Sigma-Aldrich, USA), and 5% methanol (Optima LC/MS grade, Fisher Scientific, Canada) and was pH adjusted to 5.25 using 10% HNO_3 (Optima grade, Fisher Scientific). The mobile phase was filtered through a 0.45 µm membrane and sonicated for 10 minutes before use. The injection volume for all standards and samples was 50 µL and the mobile phase flow rate was set at 1.2 mL/min. The total run time for each injection was 8 minutes.

Anion exchange separation was performed using a PRP-X100 anion exchange column (150 x 4.1 mm, 5 µm particle size, Hamilton, USA) along with a guard column (PRP-X100, Hamilton, USA) and kept at ambient temperature. Gradient elution was performed using two mobile phases: mobile phase A was composed of 60 mM ammonium bicarbonate (NH_4HCO_3) (BioUltra, Sigma-Aldrich, USA), 5% methanol and was pH adjusted to 8.2 using 10% ammonium hydroxide (Optima, Canada); while mobile phase B was composed of 5% methanol in deionized water. The mobile phase solutions were filtered and sonicated as previously described. The injection volume for all standards and samples was 30 µL and the total run time was 11 minutes for each injection. The gradient elution program is shown in Table 3.2.2.

Table 3.2.3 Gradient elution conditions for anion exchange HPLC separation

Time (min)	A%	B%	Flow rate (mL/min)
0	50	50	1.0
1.19	50	50	1.4
1.2	100	0	1.4
7.3	100	0	1.4
7.31	0	100	1.4
9.2	0	100	1.4
9.21	50	50	1.4
11	50	50	1.4

Method detection limits (MDL) were determined using the method described by the EPA (EPA, 2017). Briefly, e-liquid matrix (30 % PG and 70% VG) was spiked with 0.10 µg/L of As^{III}, DMA, MMA and

As^{V} and analyzed repeatedly nine times. Standard deviations were calculated and multiplied by the Students' t-value at 99% confidence.

3.3 Results

3.3.1 Ion Pair HPLC

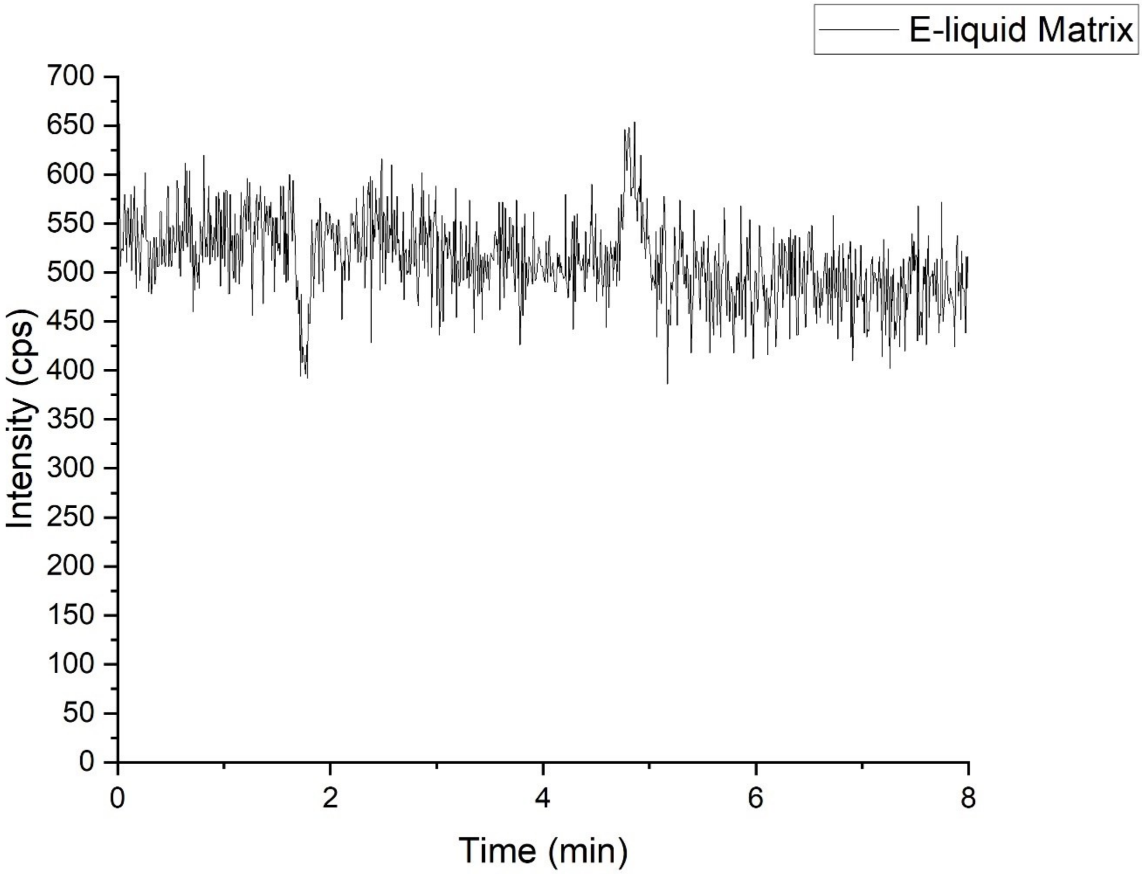

Figure 3.3.1 Ion pair chromatogram of e-liquid matrix (30 % PG and 70% VG) diluted 1:1 with deionized water, and used as the method blank

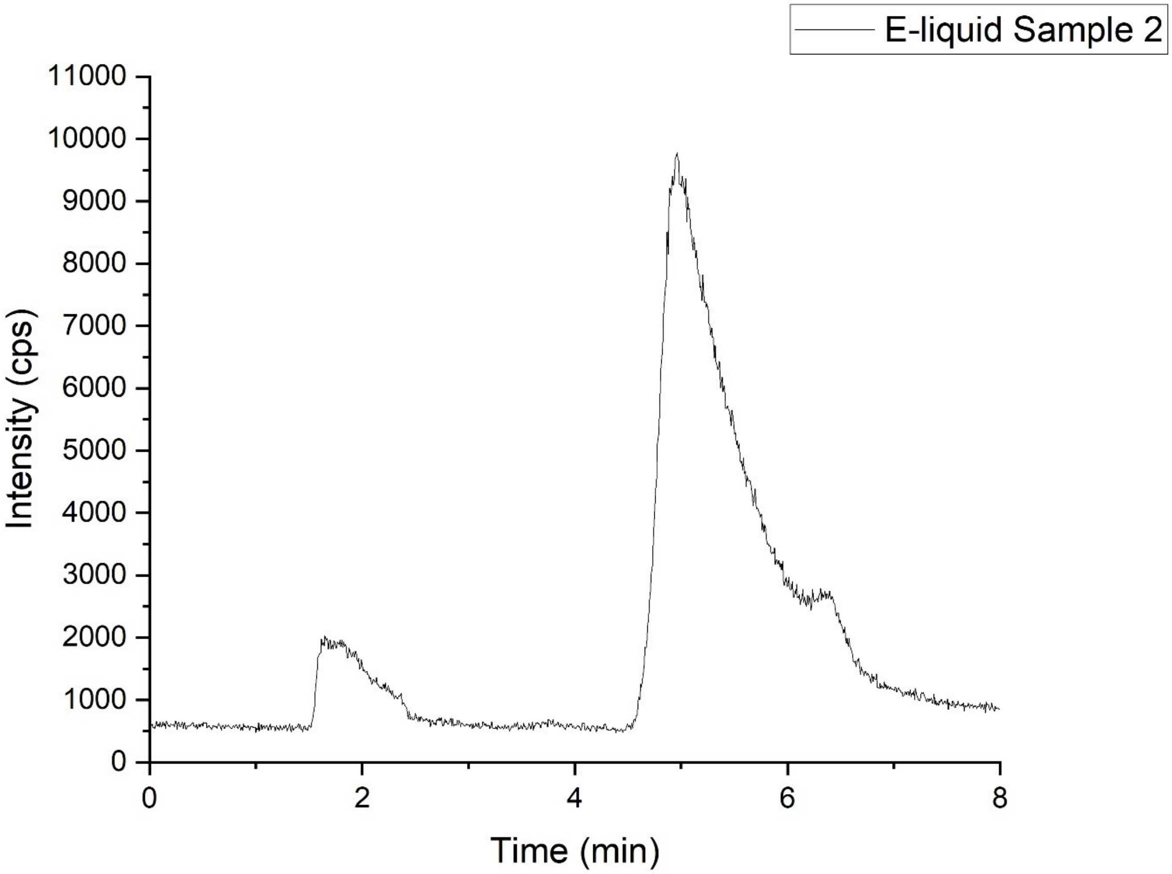

Figure 3.3.2 Ion pair chromatogram of Sample 2

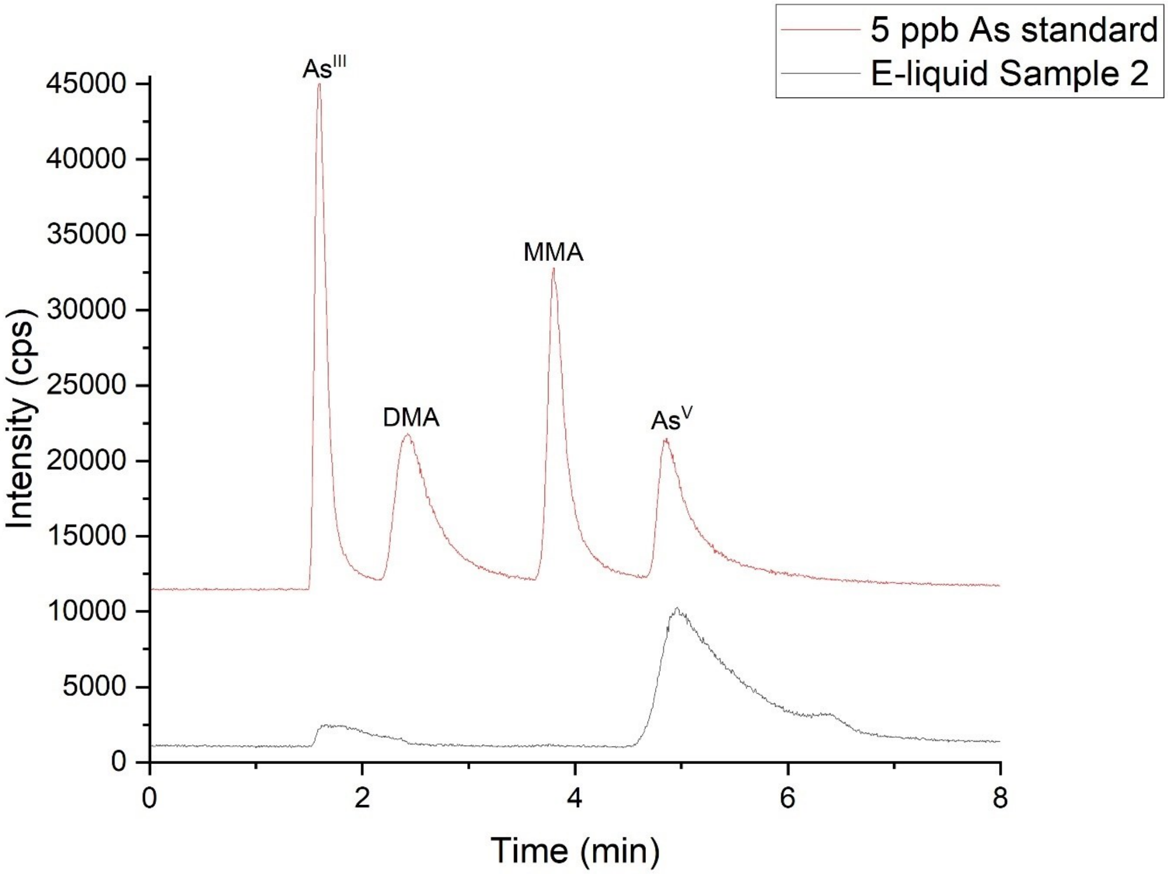

Figure 3.3.3 Ion pair chromatogram of Sample 2, and a standard containing 5 µg/L (ppb) of each arsenic species: As^{III}, DMA, MMA, and As^{V}.

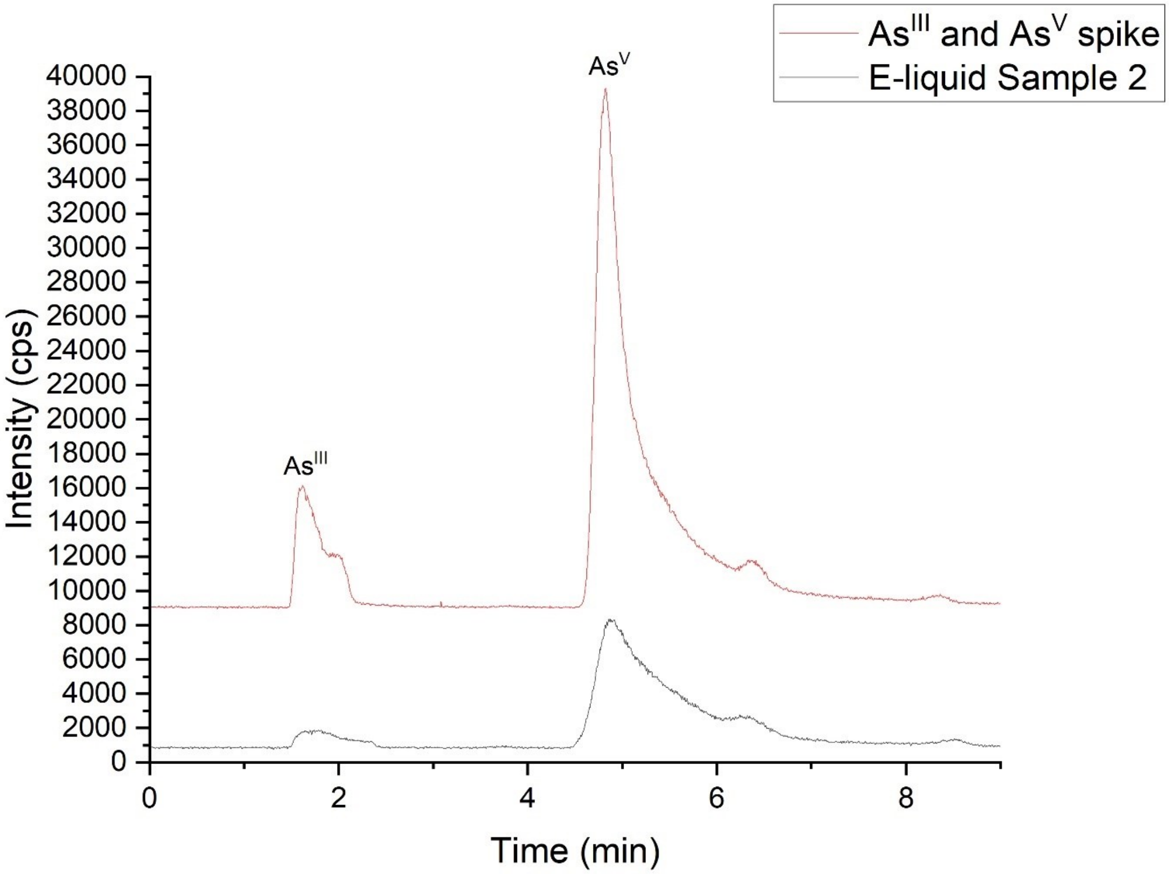

Figure 3.3.4 Ion pair chromatogram of Sample 2, and Sample 2 spiked with 3 µg/L (ppb) of As^{III} and 16 µg/L (ppb) of As^{V}

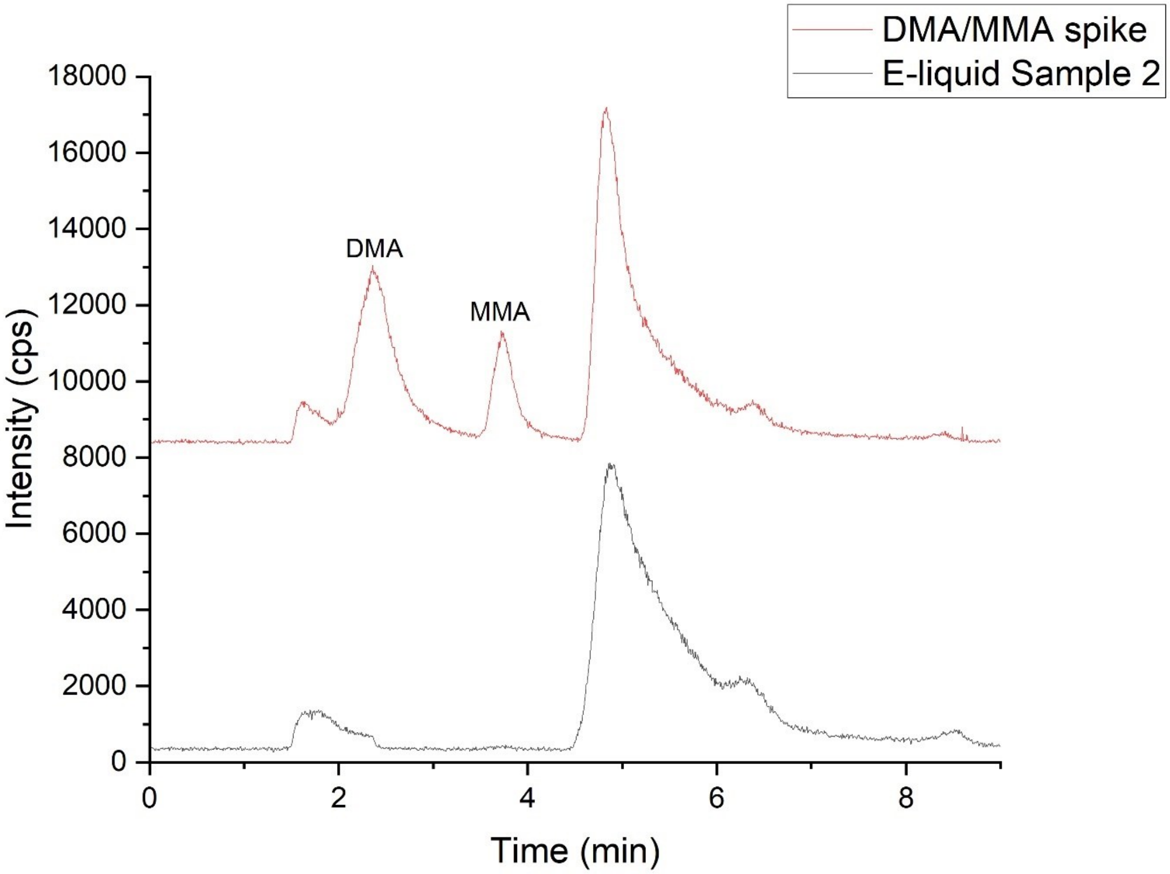

Figure 3.3.5 Ion pair chromatogram of Sample 2, and Sample 2 spiked with 3 µg/L (ppb) of DMA and 1 µg/L (ppb) of MMA

Table 3.3.1 Estimated recoveries of the spiked arsenic species from the ion pair HPLC-ICP-MS analysis

	Percent (%) recovery of As^{III}	**Percent (%) recovery of DMA**	**Percent (%) recovery of MMA**	**Percent (%) recovery of As^{V}**
Sample 1	52.9	115	97.5	98.7
Sample 2	64.5	112	115	95.0
Sample 3	62.3	120	123	91.9
Sample 4	60.9	71.6	96.5	101
Sample 5	70.9	84.5	90.3	103
Sample 6	164	-0.5	102	106
Sample 7	85.5	-0.5	102	76.9
Sample 8	73.1	127	130	56.7

Table 3.3.2 Estimated concentrations (µg/kg) of four arsenic species, As^{III}, DMA, MMA, and As^{V}, in eight e-liquid samples analyzed using ion pair HPLC-ICP-MS

	As^{III}	**DMA**	**MMA**	**As^{V}**
Sample 1	1.8 ± 0.7	0.63 ± 0.05	0.01 ± 0.01	3.7 ± 0.1
Sample 2	1.14 ± 0.02	0.30 ± 0.02	0	19.9 ± 0.3
Sample 3	0.84 ± 0.01	0.20 ± 0.03	0.01 ± 0.02	8.6 ± 0.2
Sample 4	1.74 ± 0.06	0.63 ± 0.05	0	18.21 ± 0.09
Sample 5	0.07 ± 0.03	0.07 ± 0.06	0	19.4 ± 0.2
Sample 6	1.3 ± 0.5	0.4 ± 0.1	0	14.1 ± 0.5
Sample 7	3.8 ± 0.2	1.4 ± 0.3	0	27.6 ± 0.1
Sample 8	1.37 ± 0.06	0.3 ± 0.2	0	0.55 ± 0.09
Tentative ID’s based on matching retention time with spiked sample				

Table 3.3.3 Comparison of total arsenic concentration (µg/kg) obtained using ICP-MS analysis with sum of arsenic species concentration (µg/kg) obtained using ion pair HPLC-ICP-MS analysis

	Total arsenic concentrations (µg/kg)	**Sum of arsenic species concentration (µg/kg)**	**Sum of arsenic species over total arsenic concentration (%)**
Sample 1	5.9 ± 0.7	6.9 ± 0.9	118
Sample 2	18.2 ± 0.6	24.8 ± 0.4	136
Sample 3	7.4 ± 0.3	10.4 ± 0.2	141
Sample 4	16.7 ± 0.2	20.6 ± 0.2	123
Sample 5	16.6 ± 0.1	22.2 ± 0.6	134
Sample 6	16.2 ± 0.2	18.2 ± 1.0	112
Sample 7	36.4 ± 0.7	42.1 ± 1.5	116
Sample 8	5.5 ± 0.2	2.3 ± 0.2	43

Table 3.3.4 Comparison of certified and measured concentrations of standard (or certified) reference materials using ion pair HPLC-ICP-MS

Reference Material	**Element of Interest**	**Certified Concentration (µg/L)**	**Measured Concentration (µg/L)***	**Accuracy (%)**
SRM 2669 Level II	As^{III}	5.03 ± 0.95	13 ± 4	265
	DMA	25.30 ± 0.70	2.6 ± 0.8	10.3
	MMA	7.18 ± 0.56	1.2 ± 1.0	17.4
	As^{V}	6.16 ± 0.95	0.8 ± 0.3	13.6
CRM 18	DMA	36.00 ± 9.00	25.6 ± 0.1	71
*Computed average values from all 2 measurements				

Table 3.3.5 Summary of arsenic species tentatively detected using ion pair HPLC-ICP-MS

Arsenic species	**Total number of e-liquid samples analyzed**	**Average concentration (µg/kg)**	**Median concentration (µg/kg)**	**Maximum concentration (µg/kg)**
As^{III}	8	1.5	1.4	3.8
DMA	8	0.5	0.4	1.4
MMA	8	<DL	<DL	0.01
As^{V}	8	14	16	25

Tentative ID’s based on retention time only

3.3.2 Anion Exchange HPLC

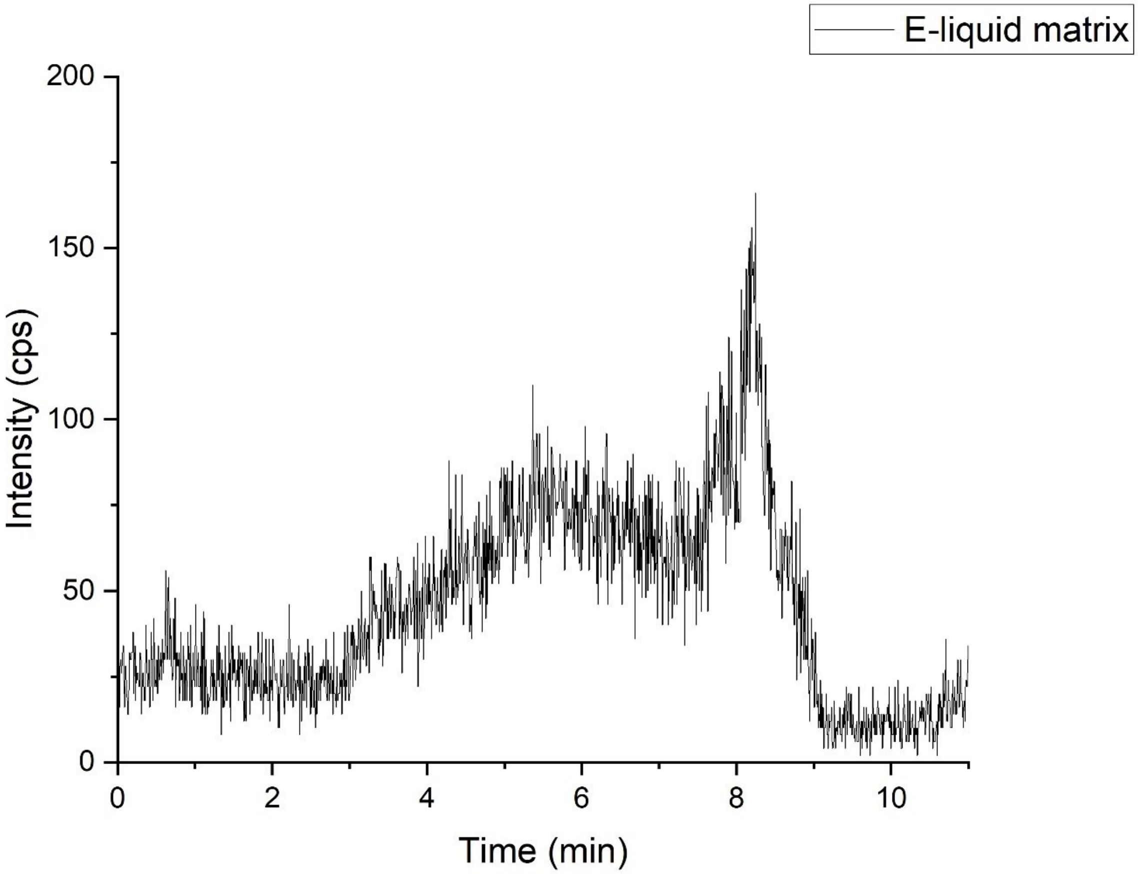

Figure 3.3.6 Anion exchange chromatogram of the method blank, composed of e-liquid matrix (30 % PG and 70% VG) diluted 1:1 with deionized water

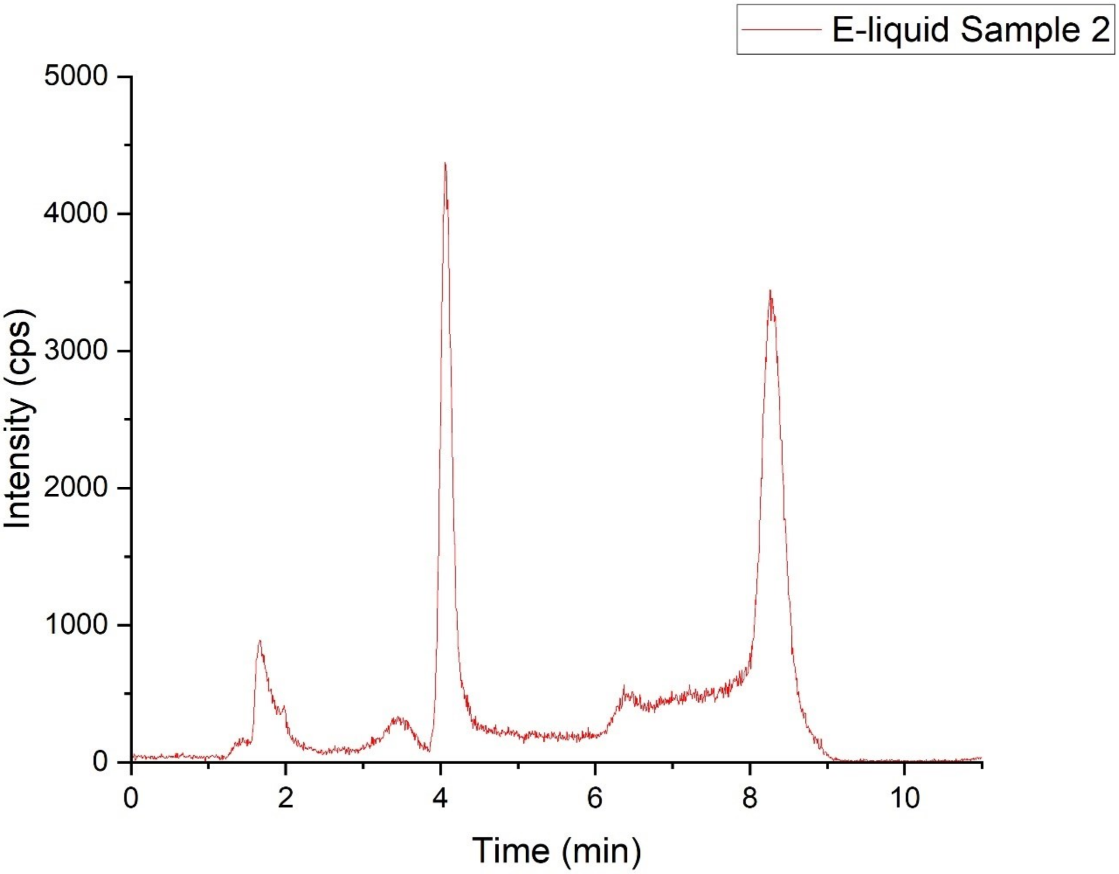

Figure 3.3.7 Anion exchange chromatogram of Sample 2

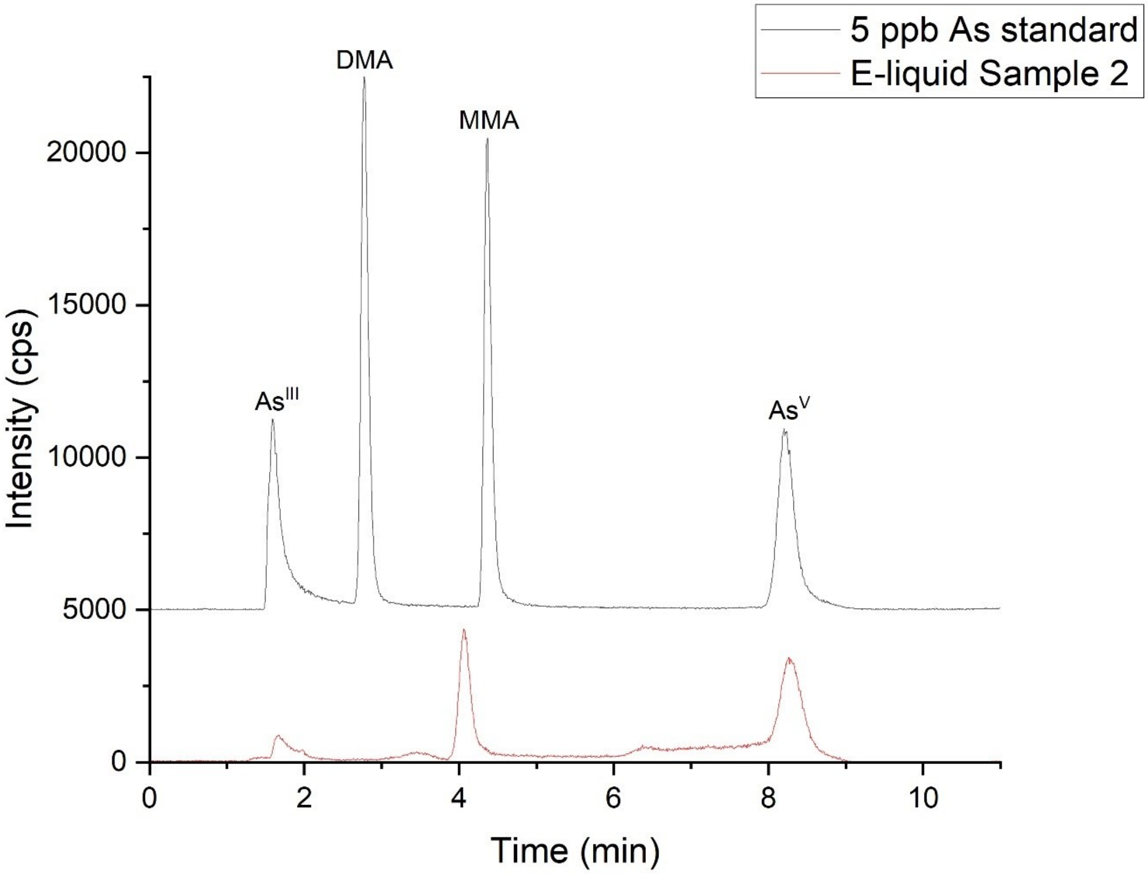

Figure 3.3.8 Anion exchange chromatograms of Sample 2 and a standard containing 5 µg/L (ppb) of each arsenic species: As^{III}, DMA, MMA, and As^{V}.

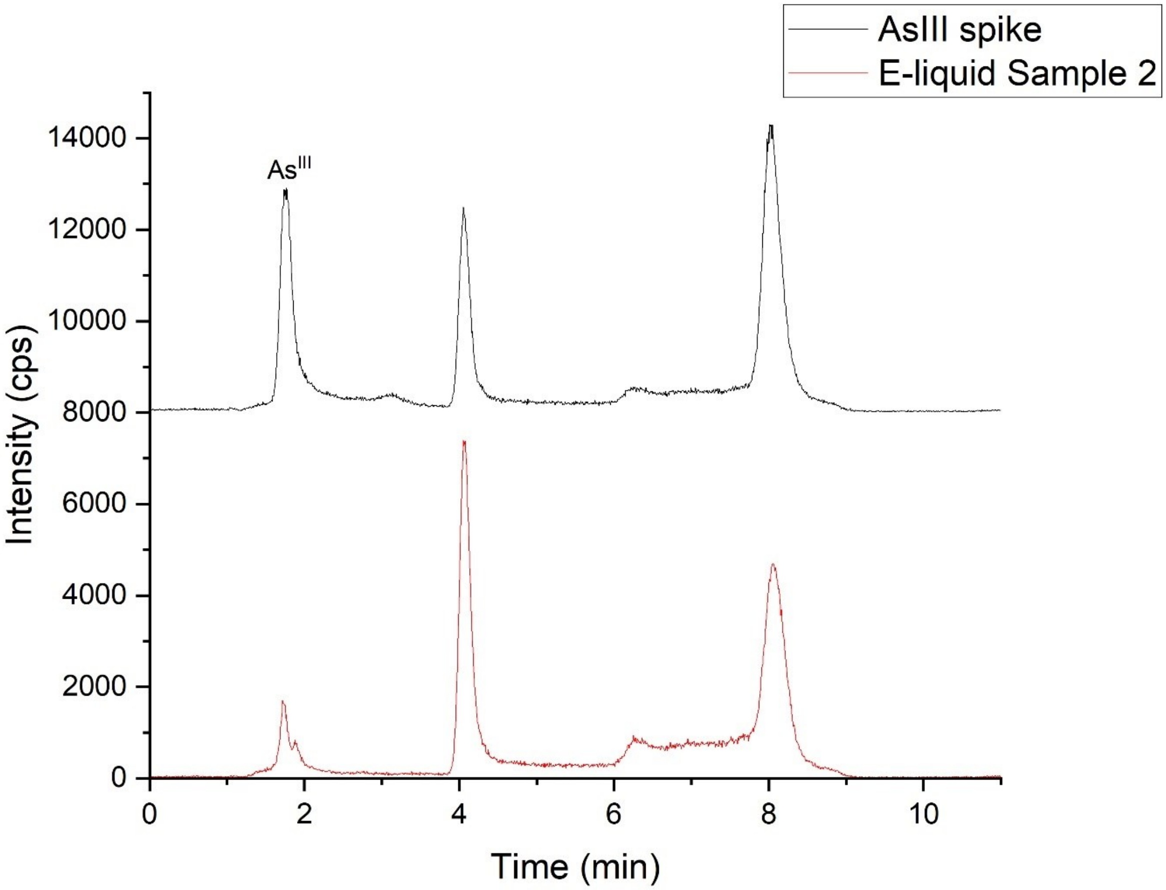

Figure 3.3.9 Anion exchange chromatograms of Sample 2, and Sample 2 spiked with 2 µg/L (ppb) of As^{III}

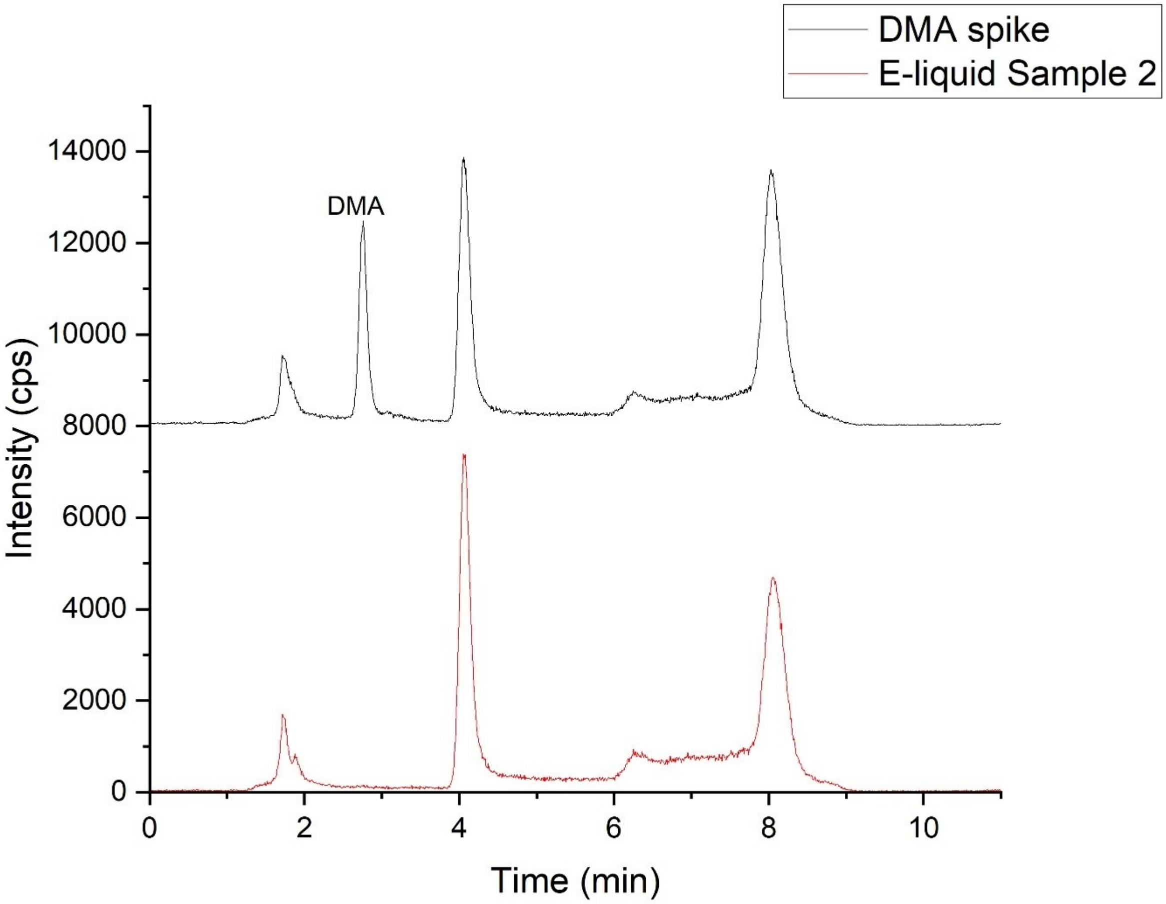

Figure 3.3.10 Anion exchange chromatograms of Sample 2, and Sample 2 spiked with 1 µg/L (ppb) of DMA

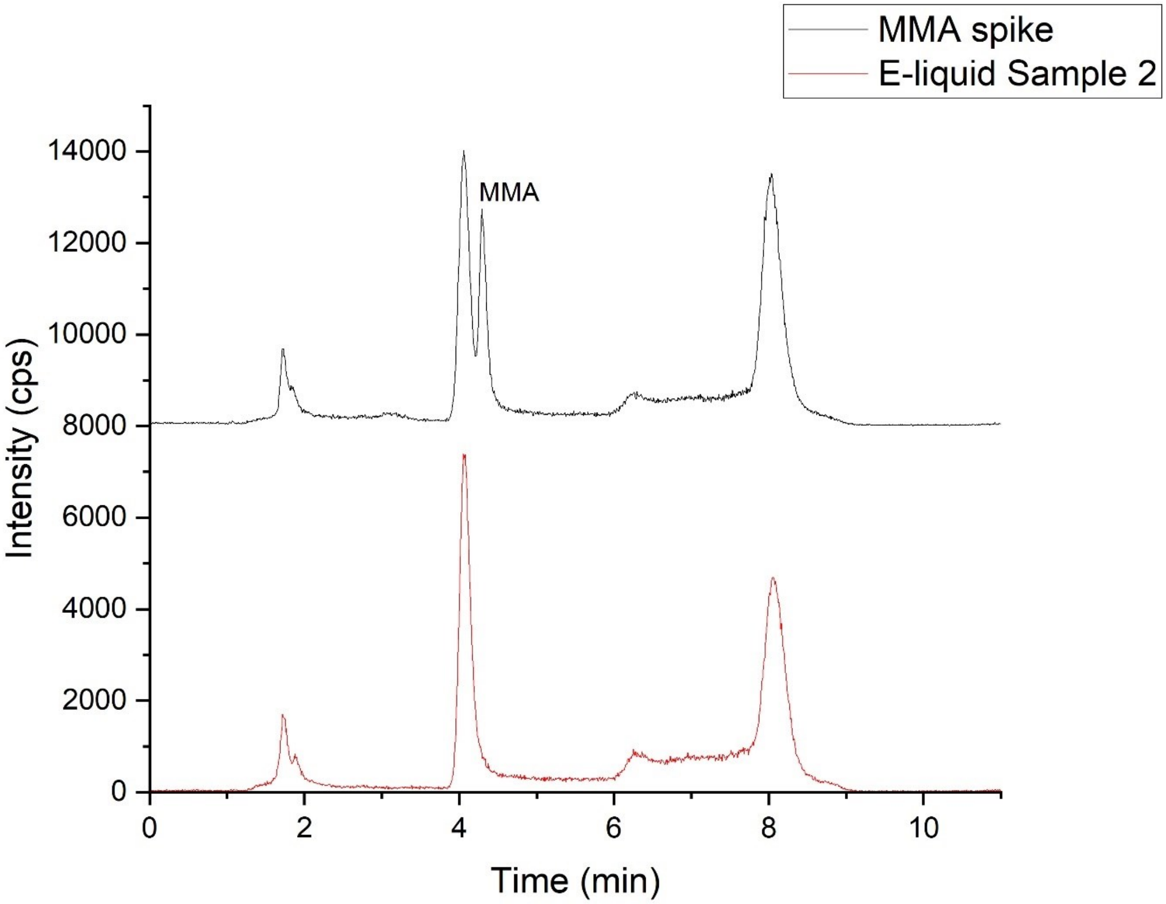

Figure 3.3.11 Anion exchange chromatograms of Sample 2, and Sample 2 spiked with 1 µg/L (ppb) of MMA

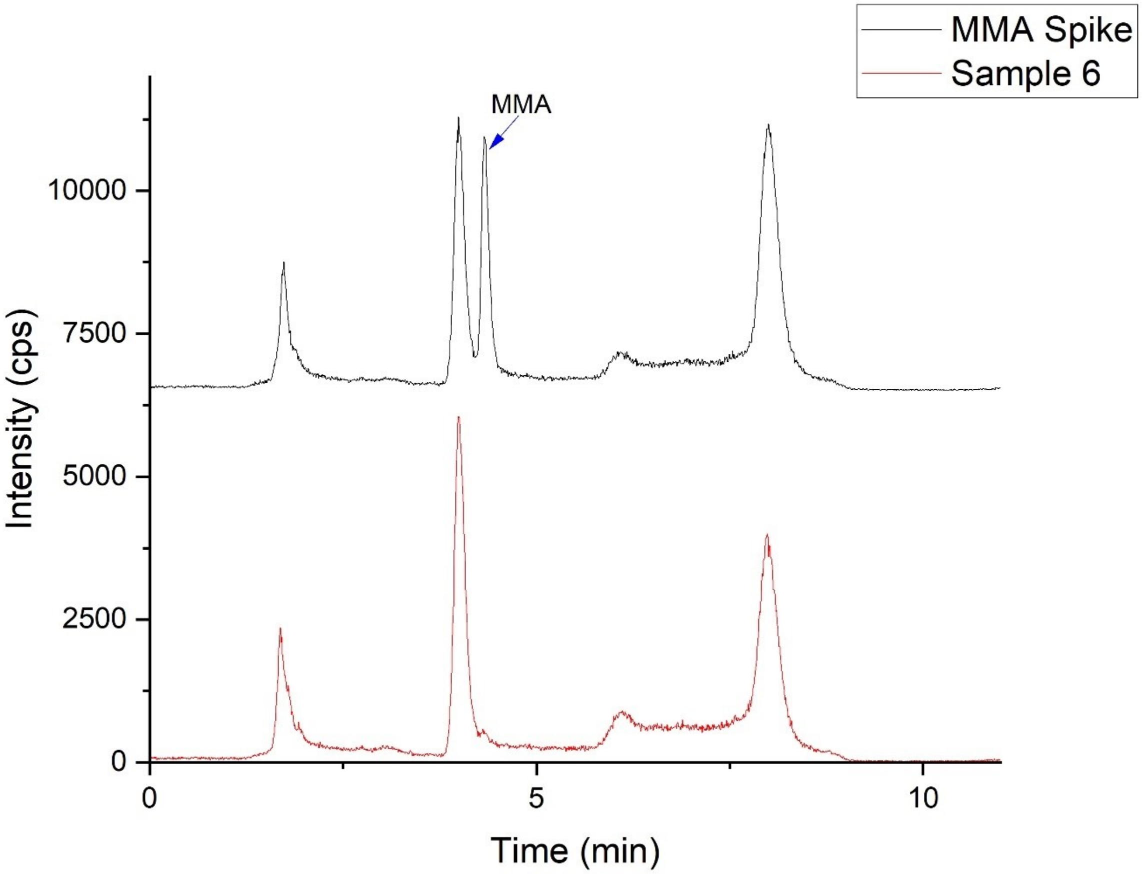

Figure 3.3.12 Anion exchange chromatograms of Sample 6, and Sample 6 spiked with 1 µg/L (ppb) of MMA

Table 3.3.6 Recoveries of arsenic species spiked to e-liquid samples. Concentrations of spiked arsenic species ranged from 1 µg/L to 8 µg/L. The samples were analyzed using anion exchange HPLC-ICP-MS

	Percent (%) recovery of As^{III}	**Percent (%) recovery of DMA**	**Percent (%) recovery of MMA**	**Percent (%) recovery of As^{V}**
Sample 2	94.7	98.9	112	N/A
Sample 3	90.3	98.1	110	45.9
Sample 4	92.5	94.8	80.0	N/A
Sample 5	82.7	103	98.9	N/A
Sample 6	92.3	97	97.8	66.2
Sample 7	100	95	110	N/A
Sample 8	62	99.8	93.5	67
N/A denotes data not available				

Table 3.3.7 Total arsenic concentration (µg/kg) obtained from ICP-MS analysis, along with concentrations of four arsenic species, As^{III}, DMA, MMA, As^{V}, and the sum of arsenic species determined using anion exchange HPLC-ICP-MS

	Total arsenic concentrations (µg/kg)	**As^{III}**	**DMA**	**MMA**	**As^{V}**	**Sum of arsenic species concentration (µg/kg)***	**Sum of arsenic species over total arsenic concentration (%)**
Sample 1	5.9 ± 0.7	2.8 ± 0.3	<DL	<DL	0.81 ± 0.09	4.4 ± 0.3	75
Sample 2	18.1 ± 0.6	1.8 ± 0.1	<DL	<DL	4.9 ± 0.3	11.2 ± 0.4	61
Sample 3	7.4 ± 0.3	1.4 ± 0.1	<DL	<DL	2.6 ± 0.2	5.5 ± 0.2	76
Sample 4	16.7 ± 0.2	1.9 ± 0.1	<DL	0.41 ± 0.04	11.8 ± 0.1	15.6 ± 0.5	93
Sample 5	16.6 ± 0.1	0.40 ± 0.02	<DL	<DL	6.3 ± 0.1	11.7 ± 0.5	71
Sample 6	16.2 ± 0.2	1.6 ± 0.1	<DL	0.15 ± 0.02	3.9 ± 0.1	10.0 ± 0.3	62
Sample 7	36.3 ± 0.7	7.5 ± 0.1	<DL	<DL	8.8 ± 0.2	25.6 ± 0.2	70
Sample 8	5.5 ± 0.2	0.92 ± 0.09	<DL	<DL	3.5 ± 0.1	5.5 ± 0.2	101
*Includes estimation of concentrations of unknown arsenic species							

Table 3.3.8 Retention times (min) and concentrations (µg/kg) of unknown arsenic species observed using anion exchange HPLC. The concentrations of Unknown 1 were estimated using the calibration of As^{III} standard. The concentrations of Unknown 2 were estimated using the calibration of DMA standard. The concentrations of Unknown 3 were estimated using the calibration of MMA standard. The concentrations of Unknown 4 were estimated using the calibration of As^{V} standard

	Unknown 1		**Unknown 2**		**Unknown 3**		**Unknown 4**	
	Retention time (min)	Concentration (µg/kg)	Retention time (min)	Concentration (µg/kg)	Retention time (min)	Concentration (µg/kg)	Retention time (min)	Concentration (µg/kg)
Sample 1	--	--	--	--	4.15	0.9 ± 0.1	--	--
Sample 2	--	--	3.45	0.2 ± 0.2	4.06	3.7 ± 0.1	6.5	0.4 ± 0.7
Sample 3	--	--	3.42	0.1 ± 0.1	4.09	1.4 ± 0.1	--	--
Sample 4	1.95	0.7 ± 0.1	3.16	0.8 ± 0.5	--	--	--	--
Sample 5	--	--	3.30	0.7 ± 0.5	4.03	3.8 ± 0.1	6.25	0.5 ± 0.1
Sample 6	1.96	0.6 ± 0.1	3.31	0.2 ± 0.2	4.01	3.1 ± 0.1	6.26	0.5 ± 0.1
Sample 7	1.97	2.7 ± 0.1	3.35	0.5 ± 0.1	4.04	5.3 ± 0.1	6.25	0.7 ± 0.1
Sample 8	1.95	0.6 ± 0.1	3.27	0.6 ± 0.1	--	--	--	--

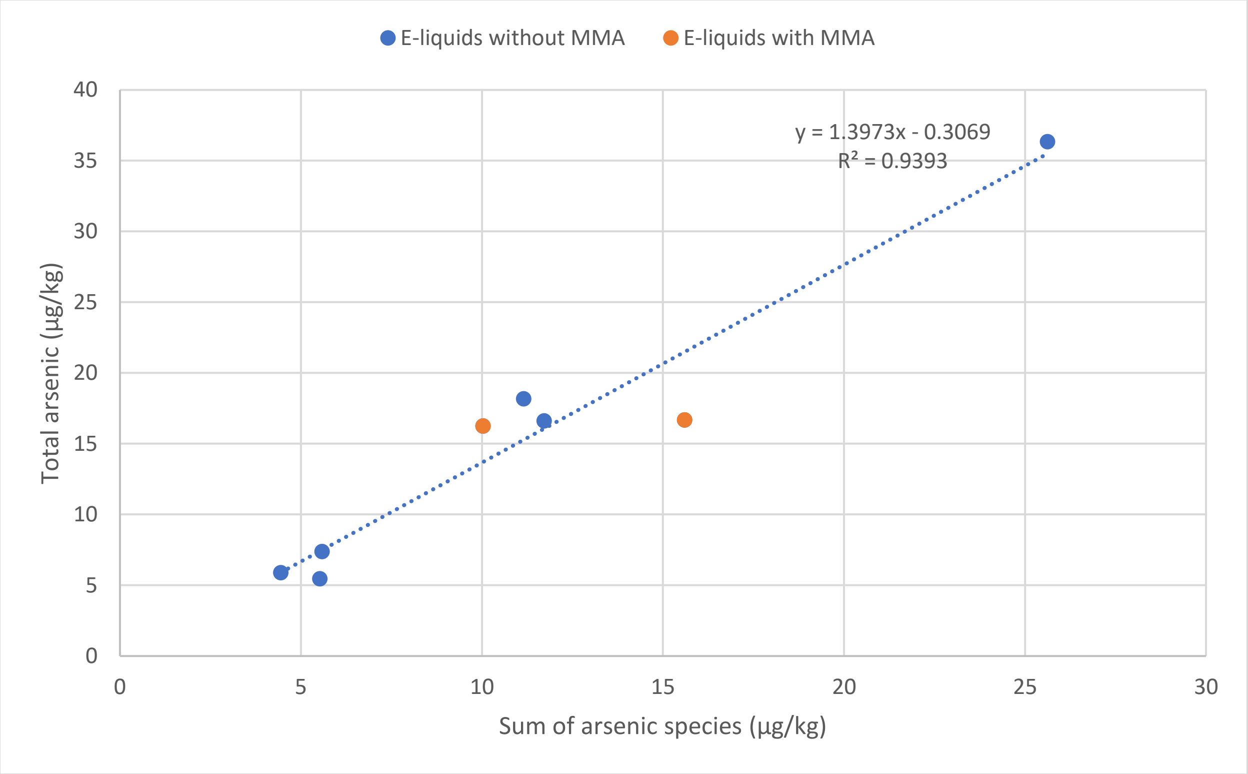

Figure 3.3.13 Total arsenic concentrations (µg/kg) obtained from ICP-MS analysis compared to the sum of arsenic species (µg/kg) obtained using anion exchange HPLC-ICP-MS. Eight e-liquids samples were analyzed using both methods

Table 3.3.9 Comparison of certified and measure concentrations of standard (or certified) reference materials using anion exchange HPLC-ICP-MS

Reference Material	Arsenic species	Certified Concentration (µg/L)	Measured Concentration (µg/L)*	Accuracy (%)
SRM 2669 Level II	As^{III}	5.03 ± 0.95	4.7 ± 0.6	94
	DMA	25.30 ± 0.70	24.3 ± 0.8	96.2
	MMA	7.18 ± 0.56	6.9 ± 0.3	95.4
	As^{V}	6.16 ± 0.95	5.5 ± 0.3	89.6
CRM 18	DMA	36.00 ± 9.00	35.4 ± 0.6	98.3
*Computed average values from all 4 measurements				

Table 3.3.10 Summary of arsenic species detected using anion exchange HPLC-ICP-MS

Arsenic species	Total number of e-liquid samples analyzed	Average concentration (µg/kg)	Median concentration (µg/kg)	Method Detection Limits (µg/L)	Maximum concentration (µg/kg)
As^{III}	8	2.3	1.7	0.10	7.5
DMA	8	<DL	<DL	0.02	<DL
MMA	8	0.07	<DL	0.02	0.4
As^{V}	8	5.3	4.4	0.04	11.8

Table 3.3.11 Method Detection Limits (MDL) of four arsenic species, As^{III}, DMA, MMA, and As^{V}, determined using anion exchange HPLC-ICP-MS. Method detection limits (MDL) were determined using an e-liquid matrix (30 % PG and 70% VG) spiked with 0.10 µg/L of As^{III}, DMA, MMA and As^{V}. Standard deviations from nine replicate analyses were multiplied by the Students' t-value.

	As^{III} (µg/L)	**DMA (µg/L)**	**MMA (µg/L)**	**As^{V} (µg/L)**
Day 1	0.07	0.02	0.02	0.04
Day 2	0.12	0.01	0.01	0.04
Average	0.10	0.02	0.02	0.04

3.4 Discussion

3.4.1 Ion Pair HPLC

Ion pair HPLC with ICP-MS detection was used to determine arsenic species in e-liquids. A representative method blank, composed of diluted 1:1 e-liquid matrix, shown in Figure 3.3.1 highlights little background contamination.

Figure 3.3.2 shows a representative chromatogram of the eight e-liquids analyzed. When compared to a 5 ppb As standard, as shown in Figure 3.3.3, it shows several peaks at retention times similar to those of As^{III}, As^{V}, and potentially DMA. However, these peaks are quite broad with significant tailing and not all peaks are baseline resolved.

A sample was spiked with a mixture of As^{III} and As^{V} or a mixture of DMA and MMA, and the sample was reanalyzed with the results shown in Figure 3.3.4 and Figure 3.3.5. The results shown in Figure 3.3.4 suggest that As^{III} and As^{V} are present in the sample while the results shown in Figure 3.3.5 suggest that DMA is possibly present in the e-liquid sample. MMA was not present in this sample, but only present in two other e-liquid samples.

The recoveries of the spiked four species to each sample are shown in Table 3.3.1. Recoveries of both As^{III} and DMA varied greatly, ranging from 52.9% to 164% for As^{III} and -0.5% to 127% in the case of the DMA. Observing the peak shape of As^{III} and the retention time of the DMA spike suggests that As^{III} and DMA are, at the very least, not baseline resolved and are coeluting, which would explain why their spike recoveries varied greatly. The recoveries of spiked MMA ranged from 90.3% to 130% while the As^{V} spike recoveries varied between 56.7% to 106%. The reason As^{V} recovery was so low in sample 8 was due to the co-elution of unknown peaks making quantification difficult. The more precise recoveries for MMA make sense given that there were no unknown or coeluting peaks present around the MMA peak.

The estimated concentrations of arsenic species are shown in Table 3.3.2. As^{V} was the predominate species followed by As^{III}. The concentrations of As^{V} ranged between 0.55 µg/kg and 27.6 µg/kg, with five out of the eight samples having concentrations greater than 14 µg/kg. The concentrations of As^{III} were quite low, ranging from 0.84 µg/kg to 3.8 µg/kg, with only one sample having a concentration greater than 1.75 µg/kg. The peak coeluting with As^{III}, thought to be DMA based on retention time matching, had very low concentrations ranging between 0.07 µg/kg to 1.4 µg/kg. MMA was only detected in two of the eight samples analyzed, having peak intensities barely above background. A summary of arsenic species that were detected in e-liquids using this method are shown in Table 3.3.5, including the average, median, and maximum concentrations (µg/kg) of arsenic species in the eight samples investigated.

For seven out of the eight samples, when comparing the sum concentration of arsenic species to the total arsenic concentration, the sum of species was greater by at least 12%. This could be due to how broad the peaks were, in combination with co-eluting peaks, resulting in the sum of species being overestimated. The sum of arsenic species should never be greater than the total concentration, and when the sum is greater, factors like sample contamination and contribution from the column are the usual culprits. However, in this case when looking at the method blank shown in Figure 3.3.1, no arsenic species aside from a tiny As^V peak were detected which rules out arsenic contribution from the column. It is possible that samples could have become contaminated however this is extremely unlikely. The most likely factor contributing to the sum of species being greater than the total concentration is the method of separation itself. As previously stated, the peaks in the chromatograms were quite broad with coeluting peaks, resulting in an overestimation on the concentrations of arsenic species, and any potential unknowns which would account for the sum of arsenic species being greater than the total arsenic concentrations.

As previously stated, SRM 2669 and CRM 18 were used to verify the accuracy of the method. As shown in Table 3.3.4, the measured concentrations for SRM 2669 and CRM 18 were compared to the certified values and the results for all of them were poor. The most accurate value obtained was for DMA in CRM 18, being just over 71% accurate while the accuracy of the measured values for SRM 2669 ranged from under 18% to over 265%. This lack of agreement suggests that the concentrations of arsenic species determined are not accurate using this method. Further improvement in separation is required for the determination of arsenic species in e-liquids.

3.4.2 Anion Exchange HPLC

Due to the peak shapes and SRM/CRM failures using a ion pair HPLC method that was not fully optimized, a different mode of HPLC was selected. Anion exchange HPLC was chosen because it has been previously applied to arsenic speciation in e-liquids, with success, by Liu and colleagues (Liu et al., 2020). A representative method blank composed of e-liquid matrix, diluted 1:1 with deionized water is shown in Figure 3.3.6. This chromatogram shows the presence of a small peak at the retention time of As^{V}.

Figure 3.3.7 shows a representative chromatogram of the eight e-liquids analyzed. When compared to a 5 ppb As standard, as shown in Figure 3.3.8, it suggests the presence of As^{III}, As^{V}, in addition to potential unknowns around where MMA would elute. To check peak identities, samples were spiked individually with As^{III}, DMA, MMA and a select few were spiked with As^{V}. Figures 3.3.9 to 3.3.11 show chromatograms of e-liquid sample 2 spiked with As^{III}, DMA, and MMA respectively. Upon inspection these chromatograms provided evidence that As^{III} and As^{V} were present in e-liquids, in addition to an unknown that elutes before MMA. Figure 3.3.12 shows Sample 6, one of the two samples that had MMA, and Sample 6 spiked with MMA standard. This highlights that the amount of MMA spiked in the samples was sufficient to account for the concentrations of MMA that were present.

The spike recoveries are shown in Table 3.3.6. The spike recoveries for As^{III} were reasonably good, ranging from 82.7% to 100% excluding sample 8, which only had a As^{III} recovery of 61.9%. Spiking recoveries for both DMA and MMA were very good, having recoveries range between 94.8% to just over 103% and 80% to 112% respectively. Recoveries of spiked As^{V} were not as good, having recoveries of 45.9% to 67%, possibly due to the increased background leading up to the As^{V} peak.

Table 3.3.7 shows the summary of the four arsenic species investigated in the eight e-liquids. As^{III} and As^{V} were found in all samples, in higher concentrations than DMA and MMA. As^{V} was the species found to have the highest concentration in all samples, ranging from 2.6 µg/kg to 11.8 µg/kg, with the exception of sample 1. As^{V} concentration in sample 1 was 0.81 µg/kg, which was lower than As^{III} concentration of 2.8 µg/kg. MMA was only detected in two samples, with concentrations below 0.5 µg/kg. DMA was not detected in any of the eight e-liquid samples. Additionally, four unknown arsenic species were also present in the samples analyzed. These four arsenic species of unknown identity are shown in Table 3.3.8 with their respective retention times, and estimated concentrations. The concentrations (µg/kg) of these unidentified arsenic species were estimated using the calibration curve information of the four arsenic standards based on proximity in retention times. The concentration of Unknown 1 was calculated using the As^{III} calibration curve; the concentration of Unknown 2 was calculated using the calibration of DMA; the concentration of Unknown 3 was calculated using the calibration of MMA, and the concentration Unknown 4 was calculated using the calibration of As^{V}. Unknowns 1, 2, 3, and 4 were detected in 50% (4 out of 8 samples), 88% (7/8), 75% (6/8), and 50% (4/8) respectively.

The sum of arsenic species, including estimations on the unknown peaks, was compared to the total arsenic concentration as shown in Table 3.3.7. Apart from sample 8, the sum of arsenic species were lower than the total concentrations. One possibility is that other unknown arsenic species could be present but not detected. These other unknown species could possibly include TMA, TMAO, or arsenolipids or arsenosugars given that the sample matrix is primarily composed of PG and VG. Figure 3.3.13 shows the relationship between the total arsenic concentration and the sum of arsenic species with a slope of 1.397 demonstrating that anion exchange can determine, on average, about 60% of the arsenic species present in these eight e-liquids.

As previously stated, SRM 2669 and CRM 18 were used to check the accuracy of the method. These results are shown in Table 3.3.9. For SRM 2669, all values fell within the certified range except for DMA where the measured value only differed by less than 4% of the certified value. For CRM 18, DMA was well within the certified value and only differed by about 1.7%. This strong agreement provides support for the quantification of arsenic species using this anion exchange method.

Method detection limits were determined using an EPA method (EPA, 2017) and the results are shown in Table 3.3.11. E-liquid matrix composed of 70% PG and 30% VG was diluted 1:1 with deionized water and spiked with 0.1 µg/L of all four arsenic species. This spiked solution was analyzed repeatedly nine times and the standard deviations from these measurements were multiplied by the Students' t-value of 2.896 at 99% confidence (n = 9).

3.4.3 Comparisons of the two methods

There were some big differences between the two separation methods used. In principle, ion pair HPLC has the capability to separate anionic, cationic, and neutral analytes all in the same analysis depending on the ion pairing reagents added to the mobile phase (Kaňa et al., 2020; Morita et al., 2007; Nan et al., 2018). Even though ion pair HPLC as been previously used for arsenic speciation analysis, it has not yet been applied to e-liquids and thus was not fully optimized. Comparing the peak shapes between ion pair and anion exchange HPLC in e-liquids, the ion pair method is poor at separating arsenic species in e-liquids while anion exchange is a better choice. Furthermore, the measured values obtained for SRM 2669 and CRM 18 were very poor. All the values obtained with the ion pair HPLC method were outside the reference values of SRM 2669. Only one value was within certified values for CRM 18. Based on retention time matching between the DMA spikes and the samples, it was thought that DMA was present however when looking at the anion exchange results, DMA was in fact not present in any of the eight samples analyzed. Additionally, MMA was not detected in any of the samples using ion pair HPLC, but Sample 4 and 6 had detectable MMA concentrations, 0.41 µg/kg and 0.15 µg/kg respectively, using anion exchange. Because the MMA concentrations were low and considering the background noise when comparing the baseline between Figure 3.3.1, which is around 500 cps, and Figure 3.3.6, which is around 15-75 cps, it is very likely that the reason why MMA was not detected using the ion pair HPLC method was because the background was too high.

These findings demonstrate that arsenic speciation analysis of e-liquids can be challenging as e-liquids are viscous samples making it difficult to handle and requires dilution to be analyzed. The target analytes, being arsenic species, are already in low concentrations and dilution further lowers the concentration, increasing the difficulty for quantification. Moreover, even with diluting the e-liquids, sample matrix remains viscous as shown in Figure 3.3.2 and Figure 3.3.7. Despite these challenges HPLC-ICP-MS was attempted by using two different separation methods; ion pair was used first in anticipation of a variety of different species being present. However due to the challenges mentioned above, in addition to the ion pair method not being fully optimized for e-liquids, ion pair was unsuccessful in achieving sufficient separation for quantification.

3.4.4 Perspectives

Previous work done by Liu et al. investigated arsenic species in seventeen e-liquids and corresponding eight e-liquid aerosols (Liu et al., 2020). The aerosols were generated using a previously established protocol (Olmedo et al., 2016) with minor modifications and a puffing topography that generates the same volume of puffed air per minute equivalent to an average slow e-cigarette user (Talih et al., 2015). Under these conditions, Liu et al. was able to calculate the concentration of inorganic arsenic (As^{III} + As^{V}) in the air (µg/m^3) from the concentration of inorganic arsenic found in the e-liquid aerosols they generated (Liu et al., 2020). The inorganic arsenic concentrations in the eight e-liquids that were selected for aerosolization ranged between 0.71 µg/kg to 3.89 µg/kg and after aerosolization the inorganic arsenic concentration in the corresponding aerosols was between 0.84 µg/kg to 5.45 µg/kg.

When converted to inorganic concentrations in the air, this corresponds to concentrations between 0.63 µg/m^3 to 4.09 µg/m^3. The United States Occupational Safety and Health Administration (OSHA) set a permissible exposure limit (PEL) of 0.010 mg/m^3 or 10 µg/m^3 over an eight-hour period to inorganic arsenic (US NIOSH, 1988).

To put the results of this thesis into perspective, the concentration of inorganic arsenic in the eight e-liquids selected for arsenic speciation analysis was 3.6 µg/kg to 16.3 µg/kg. If e-liquid aerosols were generated from these e-liquids using the same procedures and conditions that Liu et al. used, and assuming that the inorganic arsenic concentrations will not increase, which is contrary to what is shown in the literature, the concentration of inorganic arsenic in the e-liquid aerosols can be estimated to be at least 3.6 µg/kg to 16.3 µg/kg (Liu et al., 2020). Since these theoretical e-liquid aerosols were generated using the same procedure and conditions as Liu et al., the concentration of inorganic arsenic in the air are estimated to be between 2.7 µg/m^3 and 12.2 µg/m^3. When comparing these results to the OSHA permissible exposure limit of 10 µg/m^3, the upper range of 12.2 µg/m^3 is greater than the PEL set by OSHA, thus meaning that if an e-cigarette user were to vape for an eight-hour period they could be exposing themselves to inorganic arsenic concentrations in the air greater than the recommended PEL set by OSHA.

3.5 Conclusions

Both ion pair HPLC and anion exchange HPLC, with ICP-MS detection, was used to determine arsenic species in e-liquids. Ion pair HPLC was used first in anticipation of seeing more than just anionic arsenic species. However, due to not fully optimizing the ion pair method, the viscous sample matrices, the overall peak shapes, and the failure of analyzing SRM 2669, accurate quantification was not possible. Due to co-eluting peaks, the apparent matching retention times with DMA was not useful. The results from the anion exchange HPLC confirmed that DMA was in fact not present in any of the eight e-liquids. Anion exchange HPLC was then used to successfully separate and quantify three arsenic species, As^{III}, MMA, and As^{V}, and estimate the concentrations of four new arsenic species yet to be identified. Out of the four arsenic species quantified, As^{V} had the highest concentration in seven out of the eight e-liquid samples. As^{III} had the second highest concentrations among the four arsenic species. When compared to literature, the concentrations of As^{III} obtained are very similar to what has been previously reported, which is higher than MMA concentrations and lower than As^{V} concentrations overall (Liu et al., 2020). Four unidentified arsenic species were also present in the samples analyzed, with Unknowns 1, 2, 3, and 4 being detected in 50%, 88%, 75%, and 50%, respectively, of the eight e-liquid samples.

3.6 References

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Chapter 4: Conclusions and Future Work

4.1 Overview of Thesis Objectives

E-cigarettes have been around since the mid 2000's and have been used, marketed and advertised as a less harmful alternative to combustible tobacco products. E-cigarettes were marketed to aid in smoking cessation despite inconclusive evidence (National Academies of Sciences, Engineering, and Medicine, 2018). E-liquids are the solutions used in e-cigarettes. E-liquids are aerosolized and aerosols inhaled by the user. Previous research has shown that these e-liquids can be a source of exposure for contaminants, including arsenic. However there are very few studies on arsenic species in e-liquids. The overall objective of this thesis was to investigate both total arsenic concentrations, and the concentrations of arsenic species in e-liquids. In Chapter 2, I determined total arsenic concentrations, and 14 other elements such as antimony, cadmium, chromium, and lead, in 96 e-liquids using inductively coupled plasma mass spectrometry (ICP-MS). In Chapter 3, I determined the concentrations of arsenic species in selected e-liquids that contained elevated levels of arsenic.

4.2 Summary of Results

Concentrations (µg/kg) of arsenic, along with 14 other elements, were determined in 96 e-liquid samples using ICP-MS. Of the 96 samples, only 81 e-liquids had detectable arsenic concentrations, with most of them (62/81) having concentrations under 3 µg/kg. Only eight e-liquids had arsenic concentration greater than 5 µg/kg, and of those eight, five of them had arsenic concentrations between 16 µg/kg and 36 µg/kg. Other elements of concern, such as antimony, cadmium, chromium, and lead, were also detected in 88%, 15%, 47%, and 32% of the 96 e-liquids, respectively. Additionally, the various ratios of the two delivery solvents used in e-liquids, propylene glycol (PG) and vegetable glycerine (VG), were found to have no statistical impact on the concentrations of arsenic present in the 96 e-liquids analyzed, nor did the presence of nicotine have a statistical difference on the concentrations of arsenic present.

Four arsenic species; arsenite (As^{III}), dimethylarsinic acid (DMA), monomethylarsonic acid (MMA) and arsenate (As^{V}), were determined using anion exchange HPLC-ICP-MS. Ion pair HPLC-ICP-MS was first attempted in anticipation of the presence of many different arsenic species in addition to four mentioned above. However because the ion pair method was not fully optimized, co-eluting peaks in addition to the overall peak shapes and the poor results from the ion pair HPLC-ICP-MS analysis of standard reference material SRM 2669, anion exchange HPLC-ICP-MS was used instead. Using anion exchange HPLC, As^{III}, DMA, MMA, and As^{V} were successfully separated within 11 minutes. These four arsenic species were quantified in the e-liquid samples using anion exchange HPLC-ICP-MS. Additionally, four unknown arsenic species (Unknown 1 to 4) were also detected in 50%, 88%, 75%, and 50% of the eight samples analyzed. The concentrations (µg/kg) of these unknown species were estimated using the calibration of the nearest known arsenic species. For the eight e-liquid samples selected for arsenic speciation analysis, As^{V} was the predominate arsenic species. The median concentration of As^{V} was 4.42 µg/kg. The second abundant arsenic species was As^{III}, which had a median concentration of 1.72 µg/kg. MMA was detected in two samples, and the concentration was below 0.5 µg/kg. DMA was not detected in any of the samples.

Inorganic arsenic (As^{III} + As^{V}) concentrations in the eight e-liquids were ranged between 3.6 µg/kg to 16.3 µg/kg and from these e-liquids inorganic arsenic concentrations in the air were estimated, using previous literature as described in section 3.4.4, to be between 2.7 µg/m^3 to 12.2 µg/m^3. When compared to the PEL set by OSHA of 10 µg/m^3, the upper range of 12.2 µg/m^3 is greater, meaning that if an e-cigarette user were to vape for an eight-hour period they could be exposing themselves to inorganic arsenic concentrations in the air greater than the recommended PEL set by OSHA.

4.3 Future Research

Due to the viscous nature of the e-liquid sample matrix, large dilution factors, approximately 20-fold, are used prior to analysis (Hess et al., 2017; Olmedo et al., 2018, 2021; Song et al., 2018). However, there is little data showing that such large dilution factors are needed. Work done by Beauval and coworkers mentioned that dilution factors of 5-fold or 10-fold overall did not affect signal stability, but they did not show the data associated with their observations, nor did they investigate dilution factors between 10-fold and 20-fold (Beauval et al., 2016). Using smaller dilution factors may lead to increased detection of contaminates in e-liquids and thus provide a clearer picture on what users are exposed to.

This thesis, in addition to literature, has shown the presence of unknown anionic arsenic species (Liu et al., 2020). Further research is required to identify these unknown arsenic species which could include analyzing all known and available arsenic standards, using the same separation method used in this thesis, to determine the identity of the unknown species based on matching retention times. Additionally, these unknown species could possibly be identified using a simultaneous HPLC-ICP-MS and electrospray ionization mass spectrometry (ESI-MS) method. Previously, simultaneous detection using ICP-MS and ESI-MS has been used to identify and determine unknown arsenic species in a variety of matrices (Arroyo-Abad et al., 2013; Liu et al., 2015; Nan et al., 2018; Peng et al., 2017). Other methods of separation for arsenic speciation, such as reverse phase or cation exchange HPLC, could be used to investigate any arsenic-containing hydrocarbons, arsenic-containing fatty acids, and cationic arsenic species respectively (Chen et al., 2010; Contreras-Acuña et al., 2014; Jeong et al., 2017).

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