

Elemental Analysis of Tetrahydrocannabinol and Nicotine E-Liquids Related to EVALI

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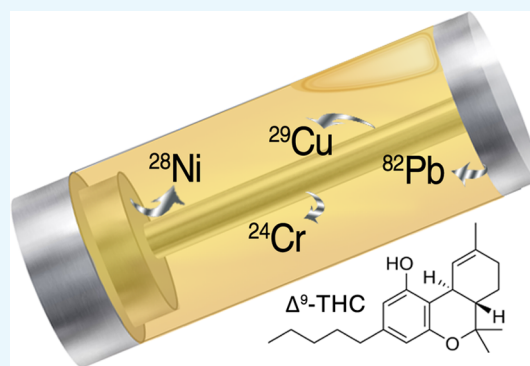


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Supporting Information

ABSTRACT: During the e-cigarette, or vaping, product use-associated lung injury (EVALI) investigation, the U.S. FDA's Forensic Chemistry Center (FCC) received numerous sample submissions from various states and other sources. Many of these products were linked directly to patients, while others were not; both categories included used and unused products. Elemental analysis using inductively coupled plasma mass spectrometry (ICP-MS) preceded by microwave-assisted decomposition was carried out on the cartridge contents of 65 of these submitted samples. Challenges encountered included limited sample, high sample viscosity, and adhesion, which necessitated sample preparation techniques not commonly used during routine elemental analysis. The elemental concentrations of contaminants including Pb, As, Cd, Cr, Ni, Cu, and Sn in tetrahydrocannabinol (THC) e-liquids associated with EVALI were determined. Nicotine e-liquid samples collected alongside the THC e-liquid samples were analyzed in tandem during method development. Several THC e-liquid samples contained Pb greater than 0.5 $\mu\text{g/g}$, while others had part per million levels of Ni, Cu, and/or Cr. This study presents the first detailed report of elemental concentrations in multiple THC e-liquid samples including those from informal/illicit sources and also delves into the method considerations needed for testing a viscous, hydrophobic sample matrix in limited quantity.



and the majority of EVALI cases, thus likely being a significant contributor to the outbreak.⁴ The initial clinical data available from small subsets of EVALI patients led to some debate about whether patients suffered from lipid pneumonia⁵ or an airway-centered chemical pneumonitis.⁶ In the early stages of the investigation, the FDA's Forensic Chemistry Center (FCC) was tasked with examining e-liquid samples for a wide range of potential chemical contaminants, including metals.⁷ Given the nature of this work, the majority of samples received by the FCC were previously used, with sample amounts ranging from trace residues to ~1 g, with generally only a few hundred milligrams available for performing all analyses. These limitations necessitated additional method development related to sample handling, preparation, and sample digestion procedures commonly utilized for elemental analysis.

INTRODUCTION

In recent years, the use of vaping devices for the consumption of tetrahydrocannabinol (THC)-containing products has increased dramatically, particularly in young adults.^{1,2} Many users reported the main reason for using a vaporizer was to reduce negative health consequences associated with smoking.³ However, in mid-2019, the U.S. Centers for Disease Control and Prevention (CDC) received numerous reports of e-cigarette, or vaping, product use-associated lung injury (EVALI). EVALI cases sharply increased in August and peaked in September before continuing to decline through February 2020. As of February 18, 2020, a total of 2807 hospitalizations have been reported from 50 states, the District of Columbia, and two U.S. territories (Puerto Rico and U.S. Virgin Islands) as well as 68 deaths.⁴ Preliminary investigations into the cause of EVALI by both state and federal agencies, including the CDC and the U.S. Food and Drug Administration (FDA), looked to determine a common cause between patient illness and the vaporized products associated with injury.

Based on the information obtained by the CDC, a total of 2022 patients (82%, as of January 14, 2020) reported using tetrahydrocannabinol (THC)-containing products and 33% claimed to use THC-containing products exclusively.⁴ This information, in combination with data produced from patient reports and product sample testing, supported a link between THC-containing vaping products from informal/illicit sources

Prior to the EVALI outbreak, heavy metal concentrations in THC-containing liquids in vaporizer cartridges and aerosols had been relatively unreported in the scientific and regulatory

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literature. However, inhalation of several elements including cadmium (Cd), cobalt (Co), manganese (Mn), mercury (Hg), nickel (Ni), zinc (Zn), and vanadium (V) has been shown to lead to chemical pneumonitis.^{8,9} The onset of symptoms of respiratory distress following exposure may not be immediate, as shown by the usual delay of several hours seen in cadmium pneumonitis.⁸ At least one literature report surmised that Co from the vaping of THC-based e-liquid (THC e-liquid) resulted in giant cell interstitial pneumonia.¹⁰ Although analytical methods were not the focus of this work; this report found 0.654 $\mu\text{g/g}$ of Co in the material from the patient's vaporizer pen. However, Co was not detected in the patient's lung samples. This work also found Ni, Mn, aluminum (Al), lead (Pb), and chromium (Cr) in the vaporizer at concentrations ranging from 0.38 to 30 $\mu\text{g/g}$. A second report¹¹ mentioned that silicon (Si), copper (Cu), Ni, and Pb levels were variable among cartridges and concentrations reached as high as 600 ppm (elemental specific results not reported). Details related to the analysis including sample preparation and quality assurance data was not included as metals analysis was only a small portion of the manuscript. Wagner and co-workers¹² performed elemental characterization of the internal components of EVALI-linked THC vaping devices using X-ray fluorescence and scanning electron microscopy.

While the information on elemental concentrations in THC e-liquids and related products is limited, there is a significant amount of recent literature on metal concentrations in nicotine-based products. These works generally focus on the concentration of potential toxic elements in the e-liquids prior to or after exposure to cartridges^{13–18} and/or in aerosols generated from vaporization of these products.^{17–23} In general, the e-liquid exposed to the cartridge, or vaporizer pen, was found to contain higher levels of some elements, suggesting that the heating coil or other cartridge-related parts, such as solder, contribute to the elemental concentration.^{18,23,24} Olmedo et al. found that the median Ni concentration in an e-liquid refill dispenser was 2.03 $\mu\text{g/kg}$ prior to exposure to a vaporizer tank and 233 $\mu\text{g/kg}$ after exposure. Also, the vapor from this solution contained 68.4 $\mu\text{g/kg}$ Ni, which was significantly higher than the e-liquid from the dispenser.¹⁸ Nicotine-based products generally use kanthal (made with iron (Fe), Cr, Al), nichrome (made with Ni and Cr), or high-purity metal (such as Ni or titanium (Ti)) coils to heat the e-liquid.²⁵ Vaporizer cartridges for THC generally consist of a ceramic-wrapped coil for heating the vape material.^{26,27} These coils can be made of a similar material as the products designed for nicotine, such as nichrome.

Cannabis sativa L. (cannabis) has been shown to be tolerant and accumulate heavy metals, prompting its proposal as a phytoremediation tool.²⁸ Cannabis grown at metal-contaminated sites had reported leaf concentrations for Cu, Cd, and Ni as high as 1530, 151, and 125 $\mu\text{g/g}$, respectively,²⁹ while another report found Cd, Ni, and Cr as high as 59, 31, and 1.2 $\mu\text{g/g}$, respectively (with 10–30 \times more in the roots).³⁰ When extracting cannabinoids, including THC, to generate THC e-liquids, a variety of procedures could be utilized. These methods have been reported in the scientific literature³¹ and on numerous websites, having varying degrees of potential for metals preconcentration in the final product. Lack of good manufacturing practices during extraction may lead to contamination of THC e-liquids with metals from various processes and/or equipment used for extraction. Many states,

including California (CA),³² have regulatory guidelines for inhaled medical cannabis products that require testing for Cd, Pb, arsenic (As), and Hg with limits of 0.2, 0.5, 0.2, and 0.1 $\mu\text{g/g}$, respectively (currently Missouri,³³ Colorado,³⁴ Maine,³⁵ and Michigan³⁶ have similar limits). These limits are derived from the International Council for Harmonization (ICH), Guideline for Elemental Impurities^{37,38} and based on the use of 10 g of the material per day as per ICH option 1 (discussed later).

It is important to note that during the course of this work, vitamin E acetate emerged as the suspected cause of EVALI.^{39–41} While metal concentrations in THC-vaporizing cartridges have not been linked to EVALI, they could have other health effects, particularly with longer-term use of THC vaporization, which makes evaluation of their concentrations in inhaled products essential. This work focuses on the methodology developed to allow for the analysis of low volumes of THC e-liquids from cartridges and the evaluation of Cd, Pb, As, Hg, Co, V, Ni, thallium (Tl), selenium (Se), antimony (Sb), molybdenum (Mo), Cu, tin (Sn), Cr, Zn, and Mn concentration in e-liquids associated with EVALI and reportedly acquired from informal/illicit sources.

RESULTS AND DISCUSSION

Digestion Procedure, Method Development. To perform metals analysis, typically 0.2–1.0 g of the e-liquid sample would be required by our laboratory. However, only a small portion (typically about 0.1 g) could be allotted for elemental analysis as the remainder of the sample was needed for other analyses to characterize these liquids as part of the EVALI investigation. Some of the sample submissions included additional unused samples not linked to a particular patient (referred herein as “research samples”); these were set aside for further method optimization and allowed for 0.4–0.7 g to be designated for elemental analysis. The initial goal was to determine if the patient-linked samples contained levels of elemental contaminants capable of causing any acute respiratory symptoms related to the EVALI crisis. Levels significantly higher than the permissible daily exposure (PDE) limits (Table 2) were of primary concern.

Using 0.1 g of the sample in total, although not ideal, allowed for multiple preparations for a basic assessment of reproducibility and analyte recovery (fortified analytical portion (FAP)) while achieving appropriate detection limits for elements of concern. As later explained, sample preparation required \sim 0.2 g of the sample, which compensated for sample adhering to pipet tips (0.025–0.075 g), stir bars, and microcentrifuge tubes. Typically, most of the remaining sample material (\sim 75%) was able to be transferred back to the original autosampler vial (\sim 30–50 mg) when using a combination of heating and centrifugation (similar to removing the e-liquid from the cartridge) to be held in reserve for future analyses.

Typically, when sampling a small portion ($<$ 0.05 g) of a free-flowing, well-mixed liquid, this portion would be assumed representative of the entire sample, as seems to be the case for nicotine e-liquids. For THC e-liquids, the sample material was not free-flowing and difficult to mix, thus potentially heterogeneous. Additionally, nicotine e-liquid samples were miscible with aqueous diluents and previous reports utilized a dilution in acid and direct analysis by inductively coupled plasma mass spectrometry (ICP-MS).^{16,42} THC e-liquids were only soluble in organic solvents for which their direct analysis

would require an instrument modification and deemed unnecessary.

To make THC e-liquids relatively free-flowing, the sample was heated to ~ 110 – 120 °C in an oven. For this purpose, we defined free-flowing as the ability to aspirate the sample with a 0.250 mL pipette tip and dispense $>75\%$ of the expected mass out of the pipette (i.e., ~ 30 mg with a 40 μ L pipet setting). Stirring the samples using a pipette tip prior to aspiration did not provide enough homogeneity (results discussed later). Attempts were made to heat the sample along with a stirring mechanism, such as a hot plate stirrer. Heating to 110 – 120 °C created problems as most plastic vials melted at this point, specifically when placed directly on the hot plate surface as the temperature on the hot plate surface was not uniform nor stable. Some glass autosampler vials were shown to leach arsenic into the samples when heated to high temperatures during stirring (>90 °C). The finalized setup was to place an aluminum heating block (designed to hold the microcentrifuge tubes and provide uniform heating) on the hot plate stirrer surface. Although the heating block hindered the magnetic stirring capability, using the maximum stir setting (1500 rpm) resulted in visibly constant stirring using a stir bar. Stir bars that were small enough for the tubes—including sizes 3–10 mm in length—were deemed adequate. Using this configuration, ~ 0.2 g of the sample in the microcentrifuge tube with a stir bar allowed for access to the liquid with a pipette tip (the opening of a 1 mL pipet was the most amenable). Using this configuration, less volume adhered to the microcentrifuge tube walls and/or stir bar and was removable via pipet. As previously mentioned, the research samples consisted of 0.4–0.7 g. The size of the stir bar was selected based on the amount of liquid in the vessel. Larger sample amounts required a larger stir bar to ensure that the entire sample volume was well-mixed (further details and results discussed later).

Using heat to decrease sample viscosity could potentially affect components in the THC e-liquid. Due to the limited sample, all characterization analyses could not be performed before and after heating to 120 °C to offer a full comparison. To assess the sample overall, using the minimal material, sample #21 was examined using Fourier transform infrared (FT-IR) spectroscopy before and after heating. No major band shifting was observed; however, minor band broadening and relative intensity differences were noted. Although only one sample was examined before and after heating and these results do not conclusively prove the absence of inter- or intramolecular conversions, major components (THC and vitamin E acetate) were clearly identified. The heating temperature is higher than 90 °C that, according to various informal websites, is commonly used to mix the THC with vitamin E and other diluents. A 120 °C environment is cooler than typical temperatures that the e-liquid encounters during the vaping process (which have been shown to be in the 400 – 500 °C range).²⁷ Given these considerations, when possible, sampling for metals analysis was typically performed after the sample was allocated for other characterization analyses so that less stable compounds of interest would not be affected.

To achieve satisfactory sample digestions, various combinations of sample mass, water, nitric acid, and hydrogen peroxide were explored. The goal was to balance these parameters with achieving complete sample digestion, diluting to yield a final acid concentration and solution volume appropriate for the ICP-MS as well as the desired detection limits. The optimized ratio was determined to be 0.025 g of the sample with 0.5 mL

of 50% (v/v) HNO_3 in water and 0.050 mL of H_2O_2 and dilution to 5 g with water after digestion. Due to difficulty in consistently weighing the THC e-liquid because of its high viscosity and adhesion, sample weights ranged from 0.010 to 0.080 g. Removing the sample material when >0.03 g was dispensed into the digestion vessel was inefficient, therefore the higher sample weights were used in these scenarios. When using the aforementioned quantities of HNO_3 and H_2O_2 with 0.040–0.080 g of the sample, the digested solution was commonly green, implying incomplete digestion. Although this did not seem to significantly impact the analysis results, it was decided to proportionally increase acid/peroxide and dilution for the higher sample weights. In general, reagent amounts/dilutions were doubled for a sample amount near 0.050 g and tripled for 0.075 g of the sample. All aspects considered, the typical sample weight range was 0.020–0.030 g. The dilution mass of 5 g provided enough liquid to be sampled twice using a standard ICP-MS CETAC autosampler.

The starting point for microwave digestion conditions using the Ultrawave was modeled after conditions in EAM 4.7⁴³ that have been shown to digest a variety of food samples, with an added intermediate heating and hold step at 200 °C to account for possible reactivity of the highly organic THC e-liquid material. The 15 mL size vessels were chosen as they were wide enough to allow placing the sample into the bottom of the tube with minimal deposits on the vessel walls. For secondary experiments (reanalysis of seven samples), a CEM microwave digestion system with the 20 mL Teflon vessels was used with the “self-venting vessels” digestion procedure described in EAM 4.7. Using this procedure, modifications were needed to yield a clear, colorless digestate. These changes included increased acid (1 mL of 50% HNO_3 and 0.100 mL of H_2O_2), dilution to 10 g, and a second heating cycle. The increased reagent volumes and additional heating cycles compensated for the lower maximum temperature of the CEM with Teflon vessels (200 °C) as compared to the Ultrawave using poly(tetrafluoroethylene) (PTFE) vessels (250 °C).

The optimized procedure utilized sample heating in a microcentrifuge tube to a maximum of 120 °C and stirred for 30–60 min with the appropriate size stir bar. Sample aliquots of approximately 0.025 g were placed at the bottom of the microwave vessel while minimizing sample deposition on the sides of the vessel. Then, 0.5 mL of 50% HNO_3 and 0.05 mL of H_2O_2 were added; within 10–15 min, all vessels were capped and placed in the microwave for digestion. The finalized digestion program using the Ultrawave was to heat to 200 °C over 20 min, then hold for 10 min, then ramp to 250 °C over 10 min, then hold for 10 min. Samples were cooled to <40 °C and transferred quantitatively with water to 15 mL centrifuge tubes, 0.025 mL of HCl (to preserve Hg) was added, followed by dilution to a final weight of 5 g (approximate dilution factor of 200) with water.

Predigestion in the presence of acid is common for other sample types, but for THC e-liquids after about 30 min the acid caused the sample to bubble and deposit the sample material on the vessel sides and cap. This could potentially lead to hot spots on the digestion vessels that could cause damage to the microwave equipment while leading to incomplete digestion, therefore predigestion was not used.

Sample Analysis. For the THC e-liquid samples, the contents of 52 individual cartridges were analyzed along with two additional THC distillates. The results are presented in

Elemental Concentrations E-Liquid Samples

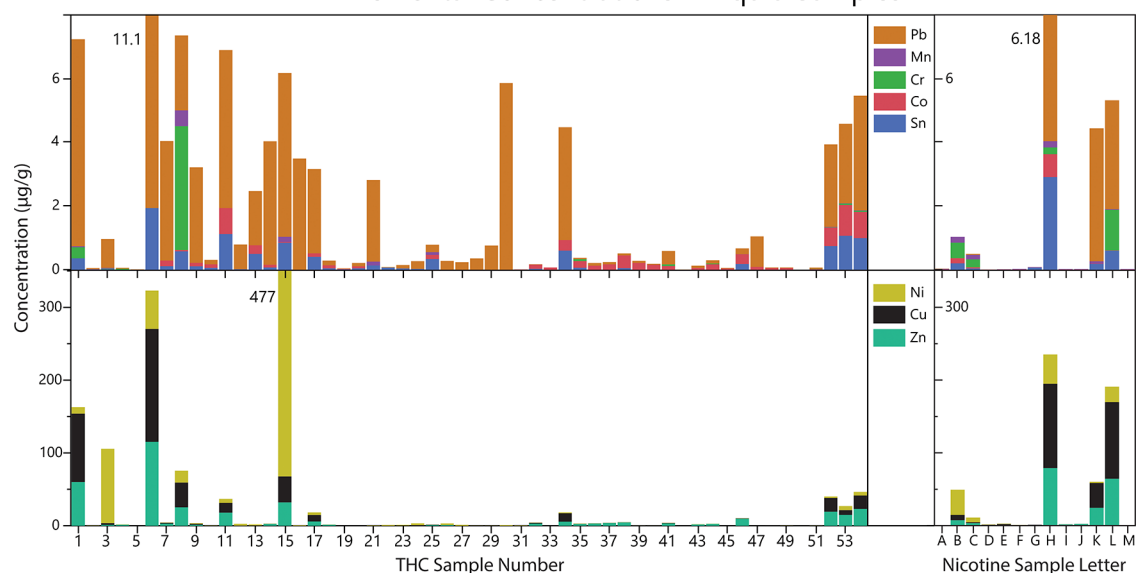


Figure 1. Elemental concentrations (Sn, Co, Cr, Mn, Pb, Zn, Cu, Ni) in THC and nicotine-related samples. Noted concentrations represent values that go off the scale. Trace concentrations (between LOD and LOQ) are represented in the stacked bar graphs without notation; for indications of such values, refer to Table S1.

Figure 1 (left side) and all elemental concentrations are reported in the text and tables as the mean \pm 2 standard deviations (2σ). The following elements in analysis groups 1 and 2 were not detected above their respective detection limits: Hg, V, Ir, Pd, Rh, Ru, and Se. The elements in analysis group 3, osmium (Os), silver (Ag), lithium (Li), and barium (Ba), were not detected in qualitative scans. A few elements were detected at trace levels (between limit of detection (LOD) and limit of quantitation (LOQ)): As, Mo, Sb, Pt, and Tl. Cadmium was only detected in one sample above the LOQ. Chromium was detected in 11 samples and only 2 were greater than LOQ (3.89 ± 0.553 and 0.348 ± 0.028 $\mu\text{g/g}$). Gold was detected in eight samples and only one was greater than the LOQ (0.930 ± 0.108 $\mu\text{g/g}$). Manganese was detected in 11 samples and only 4 were greater than the LOQ (highest = 0.495 ± 0.087 $\mu\text{g/g}$). The most prevalent elements, Co, Ni, Cu, Zn, Sn, and Pb, were detected in over half the samples and represented in Figure 1 (left side), and a complete table is present in Table S1, Supporting Information.

For the nicotine e-liquid samples, the contents of 13 individual cartridges were analyzed. The following elements in analysis groups 1 and 2 were not detected above their respective detection limits: Hg, V, Ir, Rh, Ru, Pb, Tl, and Se. The elements in analysis group 3, Os, Ag, Li, and Ba, were not detected in qualitative scans. A few were detected at trace levels (between LOD and LOQ): Cd, As, and Sb. Two elements Au and Mo were only detected in one sample each above the LOQ (2.27 ± 0.12 and 0.356 ± 0.059 $\mu\text{g/g}$, respectively). Chromium was detected in four samples greater than LOQ (1.28 ± 0.04 $\mu\text{g/g}$ was the highest). Platinum was detected in three samples and two were greater than the LOQ (highest = 0.040 ± 0.006 $\mu\text{g/g}$). The most prevalent elements, Mn, Co, Ni, Cu, Zn, Sn, and Pb, were detected in five or more samples and represented in Figure 1 (right side). A complete table is present in Table S2, Supporting Information.

A total of 17 FAPs for THC e-liquids and three for nicotine e-liquids were prepared for 22 elements in analysis groups 1 and 2. Of the total FAP data points for THC e-liquids ($n =$

374), a total of 19 recoveries were outside of the 80–120% range, with most failures due to inappropriate levels of addition or the sample irreproducibility previously discussed. Similarly, for nicotine e-liquid FAPs, all 66 recoveries were within the 80–120% range. Additionally, fortified analytical solutions (FAS) were prepared, four for THC and one for nicotine samples, with a total of six failures (outside of 90–110% of our method quality control (QC) parameters), but all recoveries were within 80–120%. Certified reference material (CRM) V23 was subjected to the same stirring/heating steps; recoveries of analysis group 1 elements V, Cr, Mn, Ni, Cu, Zn, Mo, Cd, and Pb ranged 93–115%, while Sn and Sb ranged from 47 to 113%. Based on the recovery of Sn and Sb FAPs and FASs in the THC and nicotine e-liquid samples being consistently within the criteria of 80 and 120% and 90–110%, respectively, this indicates an issue specific to the CRM. This is believed to be from the nonuniform distribution of Sn and Sb within the material, rather than matrix effects (i.e., suppression); additional work will be needed to determine the root cause.

Multiple analyzed samples exhibited % relative standard deviation (RSD) (or for $n = 2$, % relative percent difference (RPD)) of $>20\%$ (noted in Table S1). For those with this high replication error, sample homogeneity was eventually determined to be the cause. As previously explained, sample amounts were limited and the remaining material was often needed for additional testing, therefore reanalysis of these samples was rarely an option. Only one patient-linked sample exhibiting a high %RPD could be reanalyzed (sample 21). The initial analysis was sampled using only pipet tip stirring with heat and yielded %RPD ($n = 2$) for Mn, Cu, and Pb of $>160\%$. Upon reanalysis, utilizing the finalized conditions of heating to 120 $^{\circ}\text{C}$ while stirring for 30–60 min, %RSD dropped to $<20\%$, thus supporting sample heating/stirring as an important step. This trend was similar for “research samples” (non-patient-linked, unused samples #35, 44, 46, and 47). Samples #35 and #44 were analyzed multiple times using only pipet tip stirring with heat and %RSD for Ni, Zn, and Pb ranged from 3 to 59%

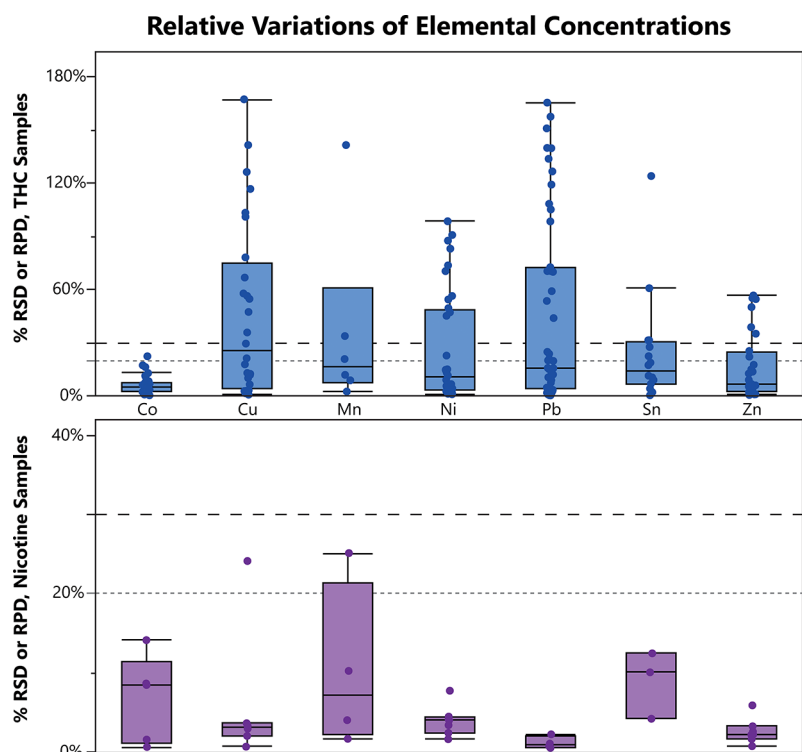


Figure 2. Relative variation of samples (%RPD for $n = 2$, %RSD for $n = 3$) for the seven primary elements detected over the LOQ in nicotine (lower panel) or THC (upper panel) e-liquid samples. Large dotted line = 30% RPD or RSD, small dotted line = 20% RPD or RSD.

and decreased in general from 4 to 27% with heating/stirring. The most significant improvement versus pipet tip only stirring was for Pb in #46 and #47, going from 25 and 140% to 4 and 20%, respectively. Four research samples, #51–54, each consisting of ~ 0.7 g, were analyzed after implementing 120 °C heating and stirring for 30–60 min initially with a small (3 mm) stir bar. During the sampling, the smaller stir bar visually appeared to only be stirring about 1/4 of the sample, and analysis yielded %RSD >20 for samples #52 (Cu) and #54 (Ni, Cu, Zn, and Sn). These two samples were analyzed again after heating and stirring with the larger stir bar (10 mm), which visibly stirred the entire sample. The %RSD did not improve for sample #52, but for sample #54 decreased to 12, 15, 14, and 16% for Ni, Cu, Zn, and Sn, respectively. However, %RSD for Co and Pd increased from 1 and 3% to 17 and 15%, respectively, which was still within the method requirements. This highlighted that despite thorough stirring, complete homogenization of THC e-liquids may be difficult to achieve. Ideally, the %RPD and %RSD should be less than 20% for samples with elements >LOQ. A summary of the repeatability of the THC-related samples for elements >LOQ ($n = 196$) is presented in Figure 2 (top portion).

By comparison, 13 nicotine samples were analyzed using the same equipment (without heating and stirring) and are summarized in Figure 1 (right side) and Table S2. The % RSD was less than 30% (only two >20%) for all elements >LOQ ($n = 38$) in all samples analyzed. This supports the conclusion that decreased sample viscosity of nicotine versus THC e-liquids plays a large part in the analysis reproducibility (shown in Figure 2, bottom portion). Reproducibility met quality control requirements for CRM V23 (with a viscosity similar to that of nicotine e-liquids) in which the %RSD for all elements were <6%, except for Sn and Sb with failing recoveries (outside of 80–120%), which were <30%.

When exploring the irreproducibility issue, one area initially considered was sample contamination during preparation, as theoretically spot contamination (from lab environment, preparation equipment, etc.) of the various elements such as Cu, Ni, and Pb, in particular, could be the cause. For example, the replicate Pb concentrations in sample #9 were 0.202, 8.05, and 0.213 $\mu\text{g/g}$, sample #13 were 4.78, 0.248, and 0.0476 $\mu\text{g/g}$, and sample #16 were 6.79 and 0.191 $\mu\text{g/g}$. For these high replicates (8.05, 4.78, and 6.79 $\mu\text{g/g}$), the corresponding solution concentrations analyzed by the ICP-MS for Pb were 20–40 ng/g (based on 200 \times dilution factor). However, when analyzing over 90 method blanks that were prepared using the Milestone system (primary system used for all reportable values for THC and nicotine-related samples), the highest method blank for Pb was 0.04 ng/g, which would correlate to a sample concentration of 0.008 $\mu\text{g/g}$, therefore, implying that spot contamination was not the cause. Trends were similar for other elements including Cr, Co, Ni, Cu, Zn, and Sn, as the maximum method blank solution concentrations were 0.3, 0.01, 0.5, 0.2, 1.4, and 0.02 ng/g, respectively. For elements with the highest levels in the method blanks, Ni and Zn (0.5 and 1.4 ng/g), the corresponding sample concentration equivalents were 0.1 and 0.28 $\mu\text{g/g}$, respectively. Therefore, the method blank contamination was not significant enough to have caused the >1 $\mu\text{g/g}$ increase for one replicate over the other replicates, which resulted in the high %RSD, as shown in Table S1. Additionally, the elements that exhibited high %RSD or %RPD were not always the same, which would require the unlikely scenario of multiple sources of single element contamination sources in separate preparations. For example, for sample #9, when compared to the other two preparations replicates, replicate 1 had high Ni, Cu, and Zn, while replicate 2 had high Pb, therefore sample heterogeneity was more likely the cause of this issue based on the observations discussed

therefore the diluents (e.g., VEA) cannot be completely ruled out as sources of the elemental contamination.

As previously mentioned, there are currently no federal regulations regarding elemental concentrations in THC or nicotine e-liquids. While no states have specific regulations for nicotine e-liquids, many states including California (CA),³² which will be used as an example for comparison, have action levels for inhalable cannabis products. The CA action levels for Cd, Pb, As, and Hg are 0.2, 0.5, 0.2, and 0.1 $\mu\text{g/g}$ of the inhalable cannabis product material, respectively. When directly comparing the results from this study to CA action levels, no products had As, Cd, or Hg concentration above the limits; based on the Pb limits, 21 e-liquids had mean concentrations greater than 0.5 $\mu\text{g/g}$.

While the regulatory landscape for cannabis products is constantly evolving, in general, state regulations for inhalable cannabis products have their basis in ICH Q3D Inhalation PDEs. ICH guidance recommends that drug products with a daily intake of not more than 10 g use the "option 1" calculation for determining the permitted concentration limit, which uses 10 g for the daily amount (e.g., if the daily intake is 3 g, 10 g is still used in the calculation). Additionally, ICH Q3D guidelines do not account for transport efficiency for elements from the product to the aerosol. Therefore, the previously mentioned levels assume a consumption of 10 g/day and 100% transmission of the elements from the THC e-liquid to the aerosol.

There are two main difficulties when correlating e-liquid elemental concentrations to user exposure: (1) variable degrees of elemental transmission from e-liquid to aerosol and (2) the lack of typical consumption rates of THC e-liquid products (due to their longer market availability, nicotine e-liquid consumption rates are more defined). To our knowledge, only one such THC e-liquid to aerosol study⁴⁶ is present in the literature, and many of those for nicotine e-liquids utilized various collection methods, many of which are not appropriate for THC e-liquids. This makes comparing data among studies convoluted (explained thoroughly in a review Zhao and co-workers⁴⁷). A study by Halstead et al.⁴⁸ generalized that aerosol transport of metals in nicotine e-liquid devices ranged from below 1% up to 4.7%. A more recent study by Mallampati et al. examined a "model" THC e-liquid (referred to as cannabis concentrates⁴⁶) fortified with various metals, also observed highly variable transport of ranging from ~4% up to quantitative (near 100%) transfer, depending on the element. As previously mentioned, consumption rates of these products are not well defined, less so for THC products. Examples of nicotine e-liquid consumptions range from 1 cartridge (~1 g) consumed per day, as reported by Flora and co-workers,⁴⁹ to 20 mL per week (considered 3 g/day for calculations, Smets and co-workers⁵⁰), up to the most conservative approach of ICH Q3D option 1 at 10 g/day.

Presenting our results as μg of element/g of THC e-liquid (Table S1) allows for future evaluations to occur once more accurate parameters, including transport and consumption rates, are better understood. For Ni, the highest concentration was 477 $\mu\text{g/g}$ (#15), three others were greater than 10 $\mu\text{g/g}$ (#3, 6, and 8), and 21 were greater than 0.5 $\mu\text{g/g}$. For Pb, the highest concentration was 11 $\mu\text{g/g}$ (#6), 4 samples (#1, 6, 15, 30) were greater than 5 $\mu\text{g/g}$, and 21 were greater than 0.5 $\mu\text{g/g}$. For Cu, the highest concentration was 155 $\mu\text{g/g}$ (#6), 4 samples (#1, 6, 8, 15) were greater than 30 $\mu\text{g/g}$, and 10 were

greater than 3 $\mu\text{g/g}$. For Cr, the highest concentration was 3.9 $\mu\text{g/g}$ (#8) and two were greater than 0.3 $\mu\text{g/g}$. For Co, the highest concentration was 0.97 $\mu\text{g/g}$ (#53) and seven were greater than 0.3 $\mu\text{g/g}$. For Au, the highest concentration was 0.93 $\mu\text{g/g}$ (#6) and no other concentrations greater than 0.1 $\mu\text{g/g}$ were detected.

Although not the primary goal of the manuscript, the analysis of 13 nicotine e-liquids presented here adds to the growing dataset of metals in e-liquids in products available to consumers on the general market. For Pb, the highest concentration was 6.2 $\mu\text{g/g}$ (H) and three samples (H, K, L) were greater than 0.5 $\mu\text{g/g}$. For Ni, the highest concentration was 40 $\mu\text{g/g}$ (H), four samples were greater than 5 $\mu\text{g/g}$ (B, C, H, L), and seven were greater than 0.5 $\mu\text{g/g}$. For Cu, the highest concentration was 115 $\mu\text{g/g}$ (H), three samples (H, K, L) were greater than 30 $\mu\text{g/g}$, and four were greater than 3 $\mu\text{g/g}$. For Cr, the highest concentration was 1.3 $\mu\text{g/g}$ (L) and one other was greater than 0.3 $\mu\text{g/g}$ (B). For Co only, one sample was greater than 0.3 at 0.72 $\mu\text{g/g}$ (H). For Au, only one sample was greater than 0.1 at 2.3 $\mu\text{g/g}$ (B).

Sample concentrations of arsenic were at levels well below the CA action levels. Nicotine e-liquid sample arsenic concentrations were similar to those presented by Lui and co-workers,⁵¹ as three samples ranged from 0.011 to 0.015 $\mu\text{g/g}$ (trace (>LOD, <LOD)). In THC e-liquid samples, arsenic was detected at trace levels in 12 samples and greater than LOQ in one sample (#24) at 0.047 $\mu\text{g/g}$. Lui and co-workers examined inorganic arsenic (iAs) in nicotine e-liquid samples and the resulting aerosol and compared their results to various inhalation guidance for iAs; however, arsenic speciation was not performed as part of this study but maybe the focus of future work.

CONCLUSIONS

This work provides the first extensive report of elemental levels in THC e-liquids, particularly those from informal/illicit sources. During method development, multiple issues were encountered and addressed, ultimately leading to a method capable of analyzing various elements in the THC e-liquids from cartridges. Additionally, based on current CA action levels of 0.5 $\mu\text{g/g}$ Pb for inhalable products, 21 of 54 samples were over the limit. Multiple literature reports utilizing various criteria concluded elements in nicotine e-liquids were present above exposure guidelines; elemental concentrations in nicotine e-liquids examined in this study were similar to those found in reports referenced throughout this manuscript.

It is worth emphasizing that not all samples analyzed were necessarily used by a patient, and not all products used by a patient were analyzed, making any correlations to EVALI nonviable. Samples #42 and #50, which were the only samples to conclusively not come in contact with a vaping cartridge, contained no detectable levels of the elements examined in this study. This, along with the elemental correlations within THC e-liquid samples that were exposed to cartridges being similar to previous reports of metals in nicotine e-liquids, supports our conclusion that the cartridges are the main source of contamination, rather than the extraction/distillation during the production of the THC e-liquid. Only one THC e-liquid (#5) and three nicotine (A, F, M) samples from cartridges did not contain elemental contaminants above the LOQ. This raises follow-up questions regarding the length of storage time in the cartridge, heating of sample in a cartridge as part of use, and the quality of various cartridges. Ultimately, detecting the

Table 1. Distribution of THC and Nicotine-Type Samples^a

sample ID	submission group	research sample	type	sample ID	submission group	research sample	type
1–2	1	N	THC	A–B	3	N	nicotine
3	2	N	THC	C	4	N	nicotine
4–8	3	N	THC	D–E	7	N	nicotine
9	4	N	THC	F	14	N	nicotine
10–11	5	N	THC	G–H	15	N	nicotine
12–13	6	N	THC	I–J	16	N	nicotine
14–15	7	N	THC	K	17	N	nicotine
16–17	8	N	THC	L	18	N	nicotine
18–20	9	N	THC	M	19	N	nicotine
21–25	10	N	THC				
26–30	11	N	THC				
31–34	12	N	THC				
35–50	12	Y	THC				
51–54	13	Y	THC				

^aSome submissions included additional unused samples not linked to a particular patient (referred herein as “research samples”); these were set aside for further method optimization and allowed for the entire portion (0.4–0.7 g) to be designated for elemental analysis.

elements in THC vaping aerosol would be of great importance to fully assess the transmission to the vapor phase and subsequently the lungs. The apparent heterogeneity of the THC e-liquid would be a topic of future work; direct analysis of the material, perhaps using laser ablation ICP-MS, would provide additional information regarding this. While analysis of larger sample portions would be ideal, this was not practical for this application where the sample amount was limited for multiple reasons. Therefore, this report presents a method appropriate for assessing the level of elemental contamination present in a quantity-limited, difficult to manipulate sample matrix.

MATERIALS AND METHODS

Samples. Samples were obtained from a total of ten states (five states had more than one submission group). A total of 54 THC and 13 nicotine containing individual items were subjected to analysis, as explained in Table 1. Samples were considered part of the same submission group if they were included in the same evidence submission made by one investigator or investigation agency. For example, an investigator from a state may have collected three cartridges from one patient or all from different patients/sources and submitted them for testing at our laboratory—all such samples were considered the same submission group. Two THC samples, #42 and #50, were obtained as large bottles containing bulk THC concentrate (THC distillate); this material was purportedly used to fill cartridges labeled to contain THC e-liquid. All other samples were received as vaping cartridges/devices containing the e-liquids. The cartridges were disassembled, and the contents were transferred to glass autosampler vials using centrifugation. Due to the nature of the sample acquisition, most of the samples received for testing showed visible signs of previous use. Each sample ID represents a unique brand and/or flavor combination from an individual submission. The recovered mass of the THC and nicotine cartridge contents (e-liquid) from this work ranged from 0.2 to 1.0 g and 0.4 to 1.9 g of the sample, respectively. Samples were classified as THC or nicotine-related if THC or nicotine was identified by Fourier transform infrared (FT-IR) spectroscopy. Because reports regarding elements in THC e-liquids products are minimal (only one sample reported to our knowledge¹⁰), nicotine e-

liquids were analyzed to serve as a comparison to other current literature reports since they are more heavily represented.

Equipment, Reagents, and Standards. All water used was 18 M Ω purity (Millipore, Massachusetts), ultra-high-purity nitric acid (HNO₃) and hydrochloric acid (HCl) (Fisher Scientific, New Hampshire) were used for standard and sample preparation, ultra-high-purity hydrogen peroxide (H₂O₂, 30% v/v) (Fisher Scientific) was used for sample preparation, and trace metal-grade HNO₃ (Fisher Scientific) was used for equipment cleaning. During method development, two different microwave digestion systems were used, a Milestone UltraWave single reactor chamber (Milestone, Sorisole, Italy) with 15 mL of PTFE (15 position rack) and a CEM Mars 5 Xpress (Matthews, North Carolina) with 20 mL Teflon vessels (40 position rack), to decompose the samples. A hot plate stirrer (Fisher Scientific) was used to heat the samples while stirring with magnetic stir bars of various sizes ((2 × 5, 3 × 3, and 3 × 10 mm), Fisher Scientific).

Elemental stock standards (10–100 μ g/mL) were obtained from Inorganic Ventures (Virginia) and contained V, Cr, Mn, Co, Ni, Cu, Zn, As, Se, Mo, Cd, Hg, Tl, Pb, ruthenium (Ru), rhodium (Rh), palladium (Pd), Sn, Sb, tellurium (Te), hafnium (Hf), iridium (Ir), platinum (Pt), and gold (Au), which were traceable to NIST-certified reference materials; standards from a secondary source (Inorganic Ventures) were used as check standards. The calibration standards were prepared at the following ranges (adjust for density): Hg—0.01–2.5 ng/g, Cu and Zn—1–250 ng/g, Mn—0.5–125 ng/g, and all others—0.1 and 25 ng/g. Germanium (Inorganic Ventures) was used as an internal standard at 20 ng/g.

An Agilent ICP-MS 8800 was used in both single quadrupole and tandem mass spectrometry (MS/MS) mode, with the former being used primarily as the MS/MS provided no necessary advantages for this application. The instrument was used with helium as a collision gas and tuned based on the manufacturer's recommendations. An Agilent ASX-500 autosampler with a cover (Teledyne, model ENC-560DC) was used for sample introduction. Due to the absence of a certified reference material for elements in nicotine and THC e-liquids, the certified reference material (CRM) V23 from LGC (New Hampshire) containing Ag, Al, B, Ba, Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, Pb, Sb, Si, Sn, Ti, V, and Zn at 10 μ g/g in hydrocarbon oil was considered the most

Table 2. ICH Guidelines for Inhalation Permitted Daily Exposures (PDE) for Elemental Impurities along with Their Respective Analysis Group and Select Figures of Merit^a

Element	ICH Class	Inhalation PDE, $\mu\text{g}/\text{day}$	Analysis Group	Solution Detection Limits (ng/g)	Method Detection Limits ($\mu\text{g}/\text{g}$)	Method Quantitation Limits ($\mu\text{g}/\text{g}$)
Cd	1	3	1	0.009	0.002	0.016
Pb	1	5	1	0.027	0.005	0.046
As	1	2	1	0.023	0.005	0.040
Hg	1	1	1	0.040	0.008	0.069
Co	2A	3	1	0.011	0.002	0.019
V	2A	1	1	0.064	0.013	0.11
Ni	2A	5	1	0.24	0.048	0.41
Tl	2A	8	1	0.080	0.016	0.14
Au, Pd, Ir, Rh, Pt, Ru	2B	1	2	0.015-0.34	0.003-0.068	0.025-0.58
Os	2B	1	3	NA	NA	NA
Se	2B	130	1	0.074	0.015	0.13
Ag	2B	7	3	NA	NA	NA
Li	3	25	3	NA	NA	NA
Sb	3	20	1	0.075	0.015	0.13
Ba	3	300	3	NA	NA	NA
Mo	3	10	1	0.075	0.015	0.13
Cu	3	30	1	0.73	0.15	1.3
Sn	3	60	1	0.050	0.010	0.085
Cr	3	3	1	0.16	0.032	0.28
Zn	NA	NA	1	7.6	1.5	13
Mn	NA	NA	1	0.045	0.009	0.078

^aAnalysis group 1: all quality control measures assessed (gray highlight); group 2: less-rigorous quality control (no highlight); group 3: elements were monitored but not quantitated (no highlight). MDL are based on $\sim 3.5\sigma$ of concentration of 90 method blanks.

appropriate. E-liquids were stored in glass autosampler vials (Fisher Scientific) at room temperature and transferred to microcentrifuge tubes (Fisher Scientific) for heating/stirring. The centrifuge used for e-liquid extraction from cartridges was from Eppendorf (Hauppauge, NY) and was operated between 3000 and 5000 rpm (1800–3000 g) for 3–10 min depending on the cartridge-type.

Due to many of the target elements being ubiquitous in the environment as well as some laboratories, special care should be taken to minimize contamination. For example, minimize or eliminate the use of metal tools; we used plastic tweezers when necessary to manipulate the cartridges. Additionally, metal-free plastic tubes (high-density polyethylene for this work), pipette tips, pipettes, etc. were used exclusively in this project and microwave vessels were cleaned to the manufacturer's recommendations.

Elemental Analysis. Targeted elements were based on those listed by the International Council for Harmonization (ICH), Guideline for Elemental Impurities.^{37,38} As shown in Table 2, the ICH guidance assigns the elements into four classes (1, 2A, 2B, and 3). For the purpose of our analyses, the elements were grouped into three "analysis groups" (as shown in Table 2). Analysis group 1, designated as the primary elements of interest and subjected to full quality control (QC) requirements,⁴³ included the class 1 elements Cd, Pb, As, and Hg; class 2A elements Co, V, Ni, and Tl; class 2B element Se; class 3 elements Sb, Mo, Cu, Sn, and Cr; and Zn and Mn. Analysis group 2 elements included class 2B elements Au, Pd, Ir, Os, Rh, Ru, Se, and Ag, which were quantitated but minimum effort was made to correct QC failures as they were infrequently detected. Analysis group 3 elements included class 2B elements Os and Ag and class 3 elements Li and Ba, which were qualitatively monitored with no formal quality control.

The analytical solution detection limits (ASDL) were determined by analyzing 90 method blanks, determining the standard deviation (σ) of their elemental concentrations, then

using calculations from FDA's Elemental Analysis Manual (EAM) Method 3.2⁵² ($\sim 3.5\sigma$). Method limits of detection (LOD) assume a nominal dilution factor of 200. Analytical solution quantitation limits (ASQL), in alignment with FDA policy, were conservatively calculated as 30σ along with a corresponding method limit of quantitation (LOQ). Elements detected between the LOD and LOQ were considered trace and noted in the results as such; additionally, these values are reported to ≤ 2 significant figures (without an $\pm 2\sigma$) as by definition trace values have an increased associated uncertainty.

For quality assurance, at least one fortified analytical portion (FAP) of approximately $0.15 \mu\text{g}/\text{g}$ Hg, $15 \mu\text{g}/\text{g}$ Cu and Zn, $7.5 \mu\text{g}/\text{g}$ Mn, and $1.5 \mu\text{g}/\text{g}$ for all others elements in analysis groups 1 and 2 was included in each sample digestion batch. The FAPs were prepared by adding ~ 0.050 mL of the appropriate standard mixture to the analytical portion (~ 0.025 g) prior to digestion. Additionally, at least two method blanks were analyzed in each digestion batch. For elements whose solution concentrations were outside of the calibration range (Cu, Ni, Zn, and Pb were the most common), additional dilutions and fortified analytical solutions (when FAP levels were not appropriate compared to native sample concentrations) were prepared and evaluated. Other quality control procedures based on EAM 4.7 v1.1 (EAM 4.7)⁴³ were followed, where applicable.

■ ASSOCIATED CONTENT

SI Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsomega.1c04868>.

Tables of values showing the elemental concentrations in both THC and nicotine e-liquid samples; figure showing the sample homogenization setup (PDF)

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Notes

The authors declare no competing financial interest.

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