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Evaluation of synthetic dyes and food additives in electronic cigarette liquids:

Health and policy implications

by

Tetiana Korzun

An undergraduate honors thesis submitted in partial fulfillment of

the requirements for the degree of

Bachelor of Science

in

University Honors

and

Biology

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To beloved chemists and the physicist

*Зробити щось, лишити по собі,
а ми, нікого, — пройдемо, як тіні,
щоб тільки неба оті голюди
цю землю завжди бачили в цвітінні.
Щоб ці іси не виіерли, як тур,
щоб ці слова не виіахли, як руди.
Життя іде і все без коректур,
і як напишеш, так уже і буде.*

Ліна Костенко

ABSTRACT

Prior studies of e-liquid thermal degradants do not reflect many of the potential health hazards related to e-cigarettes. Although current studies have focused on solvents and flavoring additives in e-cigarette formulations, there have been no prior reports on the identity and levels of synthetic dye additives. Therefore, the purpose of this study was to identify and quantify these compounds to enhance understanding of the risks associated with the inhalation of colored e-liquids. Furthermore, e-liquids were subjected to thermal degradation under normal vaping conditions to quantify the sulfur oxides (SO_x) content indicating dye decomposition. The dyes were analyzed by a combination of high-performance liquid chromatography and high resolution mass spectroscopy. The thermal decomposition of dyes in vaporized e-liquid samples was studied by ion chromatography. The findings of this investigation revealed that e-liquid manufacturers added synthetic dyes in concentrations comparable to those used in the food industry. In addition, SO_x were present in the aerosolized e-liquids suggesting that dyes undergo thermal degradation. The aerosol samples contained a substantial amount of free chloride, which could be associated with a breakdown of the sucralose molecules, whose presence in the e-liquids was confirmed by nuclear magnetic resonance.

INTRODUCTION

Electronic cigarettes (e-cigarettes) may pose dangers to consumers, due to a lack of formal oversight regarding their regulation and manufacturing. In addition, the long-term effects of vaping on human health remain to be unknown. However, e-cigarettes are frequently lauded to be a healthier alternative to tobacco products. For decades, tobacco manufacturers have faced heavy advertising restrictions, in large part to avoid encouraging tobacco use among children. It is known that advertising has a positive correlation with youth cigarette smoking.¹ The bright packaging designs of e-liquids, as well as their pleasant aromas (and potentially colors), are known to be enticing to young people.^{2,3} These attractive products, readily available on the largely unregulated market,⁴ are manufactured and advertised in a relaxed regulatory environment. As a result, in recent years, e-liquid poisoning amongst children has increased by 1500%.⁵ This includes child fatalities associated with e-liquid nicotine ingestion overdoses.⁶ If appealing color and flavoring additives in e-liquid formulations remain, there are substantive health risks to our nation's youth.

The current lack of regulations allows manufacturers to add new ingredients that have no associated inhalation toxicological data. The yields of toxic aldehydes and related compounds that are produced via the thermally-induced degradation of propylene glycol and glycerol (the e-liquid solvents) are enhanced by the decomposition of additives that are often not listed as e-liquid ingredients and are considered to be the manufacturing secrets.^{7,8}

Food additives influence the consumers' perception of food flavor and flavor identity.⁹ Such additives are classified as generally recognized as safe (GRAS) for ingestion.¹⁰ However, their inhalation toxicity is unknown. Two categories of color additives used in food and drugs in the US include those certified and those exempt from certification. They are categorized based

on the US Food and Drug Administration (FDA) testing requirements.^{11,12} Certified food dyes are synthetic compounds that are widely used because of their uniform color and shelf-life stability, as well as their ability to encompass a full spectrum of colors when mixed, while not impacting or altering food taste.¹³ The synthetic dyes shown in **Figure 1** are certified by the FDA for use in food, as well as in drugs and cosmetics (FD&C). Other FD&C colorants, not shown here, are dyes that are certified for usage only in specific foods (i.e. Orange B and Citrus Red No. 2 are used to color the surfaces of sausages and oranges, respectively).¹⁴

Figure 1. Structures of synthetic dyes.

Although disclosing the identity of synthetic dyes is mandatory on the labels of foodstuffs,¹² this information is absent on the vast majority of e-liquid labels. Additionally, acceptable daily intake (ADI) values for dye inhalation are not provided by the FDA, as synthetic dyes had not been used or intended for inhalation previously.

Herein, the purpose of this study was to identify and quantify the specific coloring compounds in commercially available e-liquids in order to enhance our understanding of their potential risks associated with inhalation and to help raise awareness about the inhalation of substances originally intended for ingestion.

METHODS AND INSTRUMENTS

Materials

Three e-liquids were ordered from the manufacturer's online shop. All HPLC-grade solvents (acetonitrile, methanol and water) were from Fisher Scientific (Fisher Scientific Co., Chicago, IL, USA). Dibasic ammonium phosphate (ACS reagent, $\geq 98\%$) and potassium hydroxide (ACS reagent grade, $>85\%$, pallets) were used for HPLC buffer preparation and were obtained from Sigma-Aldrich (Sigma Aldrich, St. Louis, MO, USA). Perchloric acid (70 % aqueous solution; Acros Organics) and hydrogen peroxide (30 % aqueous solution; Sigma Aldrich, St. Louis, MO, USA) were used for absorption solution preparation in ion chromatography experiments. Analytical standards of Allura Red AC (FD&C Red 40), Sunset Yellow FCF (FD&C Yellow 6), Tartrazine (FD&C Yellow 5), Brilliant Blue FCF (FD&C Blue 1), Fast Green FCF (FD&C Green 3), Erythrosine (FD&C Red 3) and Indigo Carmine (FD&C Blue 2) were obtained from Sigma Aldrich (Sigma Aldrich, St. Louis, MO, USA). Water for sample preparation was purified using Thermo Scientific Barnstead GenPure xCAD Plus UV-TOC Water Purification System (Thermo Scientific, Waltham, MA, USA).

Sample Preparation

Samples for HPLC-DAD analysis: E-liquids were diluted with water (1:5 dilution) and sonicated for 20 minutes. The standard solutions of Allura Red AC, Fast Green FCF, Sunset Yellow FCF, Erythrosine, Tartrazine, Brilliant Blue FCF were prepared in water by dissolving the solid powders to obtain a stock concentration of 1mg/mL. Four to eight calibration concentrations were prepared from stock solutions and used for calibration curves construction with curves forced through the origin. The concentration ranges for standard

solutions were 20-80 $\mu\text{g/mL}$ for Allura Red AC, Fast Green FCF, Sunset Yellow FCF and Erythrosine, and 2.5-80 $\mu\text{g/mL}$ for Tartrazine and Brilliant Blue FCF. Each unknown and standard solution were filtered (PVDF, 0.22 μm pore size) and subjected to HPLC-DAD analysis.

Samples for ESI-HRMS validation: dye samples from three e-liquids were concentrated using SPE cartridges (Oasis HLB cartridges, Waters, Milford, MA, USA), connected to the vacuum manifold system (Waters, Milford, MA, USA) and the vacuum inlet. Cartridges were conditioned using 3 ml of methanol and 5 ml of deionized water at a flow rate of about 1 drop per minute. E-liquid samples diluted in water were transferred to the SPE cartridges. The loaded cartridges were rinsed with 5 ml of water allowing e-liquid solvent separation at a flow rate of about 2 drops per minute. The cartridges were eluted with two 3-mL methanol rinses at the same flow rate. The eluent sample volumes containing dyes were reduced by rotary evaporation (R-210 Rotavap, Buchi, New Castle, De, USA). The residues were reconstituted in 1 ml of methanol. SPE of red and blue e-liquids yielded two samples containing red and blue dyes, respectively; SPE of the green e-liquid yielded a combination of two samples of yellow and blue dyes. The standard solutions of Allura Red AC, Fast Green FCF, Sunset Yellow FCF, Erythrosine, Tartrazine, Brilliant Blue FCF dyes were prepared in water to give a final concentration of 20 Mm. Note, Indigotine was not used for ESI-HRMS analysis.

Samples for IC analysis: the vapor was generated using the electronic cigarette consisted of Tesla Invader III battery unit (Teslacigs, Shenzhen, China) with two 18650 HG2 batteries (3.7 V, 3000 mAh) (LG Chem, Holland, MI) and KangerTech SubTank Mini atomizer with horizontal kenthal coil (1.2 Ω resistance) (KangerTech, Shenzhen, China). The e-cigarette was operated at 17 W. The aerosol was drawn into a -78 $^{\circ}\text{C}$ cold trap (dry ice/acetone)

followed by the impinger containing acidified hydrogen peroxide absorption solution. The setup was connected to the SCSM-STEP single cigarette-smoking machine, simulating inhalation (CH Technologies, Westwood, NJ). Aerosol was produced using CORESTA vaping mode (3-s puff with 1-s power button activation prior vaping, 30-s puff intervals, 55-mL puff volume).¹⁵ The sample from the cold trap was combined with the impinger solution. The samples were immediately subjected to analysis by ion chromatography. Triplicate experiments were performed using each e-liquid; each replicate represents the session of 40 puffs (20 puffs – 20 minutes cooling – 20 puffs).

HPLC-DAD Analysis

The samples of commercially available e-liquids were analyzed using adapted HPLC-DAD method.¹⁶ The HPLC-DAD set-up included 1525 Binary HPLC pump, 2996 Photodiode Array Detector and column heater (Waters, Milford, MA). The chromatographic separation was performed using Acclaim PA2 column with 3 μ m particle size, 3 $\times$ 75 mm dimensions and injection volume of 5 μ L (Thermo Scientific, Waltham, MA). The DAD detector was set to 210–650 nm spectral range. The column was kept at 30 °C. The mobile phase consisted of buffers A (20 mM (NH₄)₂HPO₄, pH 8.8) and B (20 mM (NH₄)₂HPO₄: CH₃CN = 50:50, v/v).

The analysis of standard solutions was performed using the gradient of 12% B from 0.00 to 3 min, ramping to 100% from 3.00 to 3.50 min with hold for 1.0 min, and return to 12% B in 0.1 min (flow rate of 0.71 mL/min). The e-liquid samples followed the same gradient program and flow, except that the 100% B hold was extended to 29.50 min and returned to 12% in 1 min.

Table 1. Quantitative features of the HPLC-DAD method for the selected dye standards

Dye Standard	Absorption Maxima (nm)	Regression (%)	LOD ($\mu\text{g/mL}$)	LOQ ($\mu\text{g/mL}$)	RSD (%)
Allura Red AC	506	99.998	0.27	0.88	1.37
Fast Green FCF	619	99.988	0.97	3.23	2.39
Sunset Yellow FCF	485	99.972	1.60	5.35	0.36
Erythrosine	530	99.972	1.57	5.23	2.45
Tartrazine	426	99.991	0.57	1.91	0.81
Brilliant Blue FCF	630	99.973	0.10	0.34	1.49

Experiments and the construction of calibration curves were performed in triplicates.

Quantitative features of the HPLC-DAD method and chromatograms of the dye standards are presented in **Table 1** and **Figure 2** (additional chromatograms: **Supplementary information**).

Empower 2 Chromatography Data Software was used for data collection and processing.

Figure 2. Representative chromatograms of synthetic dye standards. Standards at 40 $\mu\text{g/mL}$ concentrations.

ESI-HRMS Validation

Validation of identified analytes was performed using a high-resolution (30,000 resolution power) Thermo LTQ-Orbitrap Discovery hybrid mass spectrometry instrument equipped with Ion Max source with an electrospray ionization probe (Thermo Fisher Scientific, San Jose, CA, USA). The ionization interface was operated in the negative mode using the following settings: source voltage, 4 kV; sheath and aux gas flow rates, 50 and 5 units, respectively; tube lens voltage, 90 V; capillary voltage, 49 V; and capillary temperature, 300 °C. The Orbitrap mass analyzer was externally calibrated prior to analysis. ESI-HRMS spectra of standards (molecular ions: Allura Red AC $[M]^{2-}$, Brilliant Blue FCF $[M]^{2-}$, Tartrazine $[M]^{3-}$, Sunset Yellow FCF $[M]^{2-}$, Erythrosine $[M]^{2-}$ and Fast Green FCF $[M]^{2-}$) revealed the molecular ion masses with accuracy within ± 7 ppm (**Table 2** and **Supplemental information**).

Table 2. Quantitative features of the ESI-HRMS method for the selected dye standards.

Standard	Calculated Mass of Standards (<i>m/z</i>)	Observed Mass of Standards (<i>m/z</i>)
Allura Red AC	225.00903	225.00924
Brilliant Blue FCF	373.07077	373.07067
Tartrazine	154.99315	154.99263
Sunset Yellow FCF	202.99592	202.99515
Erythrosine	834.64667	834.64394
Fast Green FCF	381.06823	381.06713

Ion Chromatography

Anion analysis of the vaped samples was conducted using an Dionex ICS-5000 ion chromatography system (Dionex, Sunnyvale, CA), outfitted with a conductivity detector cell and electrolytically regenerated suppressor (AERS 500, 4mm; Dionex, Sunnyvale, CA). 25 μ L aliquots of the filtered (0.2 μ m pore size) samples were injected onto the system for each run. The separation was carried out on an IonPac-AS15 with an IonPac-AG15 guard columns

(Dionex, Sunnyvale, CA) and a flow of 0.75 mL/min. Gradient elution was used to obtain separation of the organic and inorganic peaks. The eluent concentrations were set as follows: 3 mM KOH for 36.5 minutes, 45 mM KOH for 16.5 minutes, and 3 mM KOH for 7 minutes. Calibration curves were created using seven calibration standards with concentrations of chloride and sulfate ranging from 2.50 to 40.0 mg/L and 0.125 to 2.00 mg/L, respectively

(Supplementary Information). Data acquisition and analysis was performed using Chromeleon workstation. Linear calibration curves (without forcing the intercept through zero) were created based on peak height. R-squared values for all calibration curves were greater than 0.99; LOD values for chloride and sulfate were 0.063 and 0.079 mg/L, and LOQ values were 0.19 mg/L and 0.24 mg/L, respectively.

RESULTS AND DISCUSSION

Three e-liquids were randomly chosen to represent the primary additive colors (red, green and blue). The manufacturers were contacted but declined the requests for the identity of ingredients, including the synthetic dyes used in the formulations.

Three dyes, Allura Red AC, Brilliant Blue FCF and Tartrazine, were identified in the e-liquid samples. According to their structures, Allura Red AC and Tartrazine are azo dyes, and Brilliant Blue FCF is a triarylmethane (**Table 3**). Although these artificial food colorants are generally regarded as safe (GRAS) for human consumption, batches of Allura Red AC, Sunset Yellow FCF and Tartrazine are known to contain the human carcinogen benzidine and other aromatic amines¹⁷⁻²⁰, while Tartrazine is a known potent allergenic agent.²¹⁻²⁴

Table 3. Seven synthetic dyes used in foods. Chemical classes and ingestion ADIs.

Synthetic Dye	Chemical Class	European ADI (mg/kg/day)	ADI ²⁵ (mg/p/day) ^a	Per Capita Exposure ²⁵ (mg/p/day) ^a
Allura Red AC (FD&C Red No. 40)	Azo	0-7 (2016) ²⁶	420	17.91
Sunset Yellow FCF (FD&C Yellow No. 6)	Azo	0-2.5 (1982) ¹³	225	10.74
Tartrazine (FD&C Yellow No. 5)	Azo	0-10 (2016) ²⁶	300	12.06
Brilliant Blue FCF (FD&C Blue No. 1)	Triarylmethane	0-12.5 (1969) ¹³	720	1.72
Fast Green FCF (FD&C Green No. 3)	Triarylmethane	0-25 (1986) ¹³	150	0.038
Erythrosine (FD&C Red No. 3)	Xanthene	0-0.1 (1990) ¹³	150	0.61
Indigotine (FD&C Blue No. 2)	Sulfonated Indigo	0-5 (1974) ¹³	150	1.95

^a Per 60-kg person

The identification and quantification of the dyes in the samples was performed by high-performance liquid chromatography-diode array method (HPLC-DAD). The chromatograms of three e-liquid samples were compared to chromatograms of the standard solutions of Allura Red AC, Sunset Yellow FCF, Tartrazine, Brilliant Blue FCF, Fast Green FCF, Erythrosine and

Indigotine (**Figure 3**). The green e-liquid contained two dyes, Brilliant Blue FCF and Tartrazine, at concentrations and proportions consistent with those used in the food industry to afford neon green solutions.²⁷ The blue and red e-liquids contained Brilliant Blue FCF and Allura Red AC food colorants, respectively (**Table 4**).

Table 4. Identification and quantification of unknown dyes in the samples of commercially available e-liquids.

Sample	Identified Dye	Absorption Maxima (nm)	Retention time (min)	Concentration ($\mu\text{g/g}$) ^a
Red	Allura Red AC	505	2.38	66.71
Blue	Brilliant Blue FCF	630	3.01	51.42
Green	Brilliant Blue FCF	630	3.00	1.85
Green	Tartrazine	426	1.38	29.43

^a μg of synthetic dye per g of e-liquid

Figure 3. Chromatograms of unknown dyes.

Notice that the green sample is made of a combination of yellow and blue dyes. Detector wavelengths were set at 426 nm for Tartrazine, 505 nm for Allura Red AC and 630 nm for Brilliant Blue FCF.

Follow-up validation by high resolution electrospray ionization mass spectroscopy (ESI-HRMS) confirmed the results obtained by HPLC-DAD method. Allura Red AC, Brilliant Blue FCF and Tartrazine were identified with mass accuracies within ± 7 ppm (**Table 5** and **Supplementary information**).

Table 5. ESI-HRMS validation of unknown dyes in commercially available e-liquids.

E-liquid Sample	Dye Identified	Observed mass (<i>m/z</i>)	Calculated mass (<i>m/z</i>)
Red	Allura Red AC	225.00829	225.00903
Blue	Brilliant Blue FCF	373.06815	373.07077
Green	Brilliant Blue FCF	373.07184	373.07077
Green	Tartrazine	154.99319	154.99315

The overall data shows that manufacturers are using the three FDA certified dyes at concentrations similar to those used in the food industry (**Table 6**).

Table 6. Synthetic dye concentrations in selected foodstuffs.

Foods	Tartrazine ($\mu\text{g/g}$)	Brilliant Blue FCF ($\mu\text{g/g}$)	Allura Red AC ($\mu\text{g/g}$)
Soft drinks ²⁸	4.7-5.2	1.0-1.2	49.8
Confectioneries ²⁸	4.0-154.8	1.0-6.3	ND
Water soluble foods ²⁹	0.5	0.5-4.8	18.1-27.8
Gummy candy ³⁰	1.7-80.4	0.7-9.6	1.0-47.8
Jellies ³⁰	2.2-20.2	ND	ND
Juices ³¹	0.06-121.82	2.75-71.44	5.77-7.15
Cookies ³¹	0.13-17.36	0.90-0.99	ND
Fruit jam ³²	ND	ND	17.9-33.4
Salted fish ³²	136.0-292.5	ND	ND
Soft drinks ³³	158	0.063-12.9	0.107-0.14
Juice and jelly powder ^{34a}	1.3-56.2	2.9-6.4	30.2-53.8

^a Units for this study are $\mu\text{g/mL}$; ND = not disclosed or not discovered in specified foods.

The justification for colorant addition to e-liquids in the same proportions relative to foodstuffs is likely to enable a vaper's identification of e-cigarette flavoring additives with corresponding foods. The color was shown to influence food sensory characteristics and acceptability of products due to possible learned associations of particular colors with foodstuffs.^{35,36} Other reasons for using artificial dyes in e-liquids could include the masking of unattractive natural colors of flavorings or other e-liquid constituents, or protecting e-liquids via the sunscreen effect¹³ to extend their shelf life. Although these reasons are valid for justifying

dye addition to foods, the benefits of e-liquid color enhancement may be undermined due to the unknown health risks associated with the inhalation of dyes and their potential degradation byproducts.

Several conundrums arise regarding the lack of (i) certified dye labeling on e-cigarette packaging and (ii) acceptable daily intake (ADI) values for their inhalation. In agreement with the requirements of CFR 21, §70.25, certified synthetic dye identities are required on the labels of not only food products, but also on drugs and cosmetics.¹² Although e-liquids are intended for human consumption, the identities of any dyes used are not required to be disclosed.

More importantly, there are no inhalation ADI values for these dyes, as inhalation is just emerging as an intended method of their consumption. The European ADI values for dye ingestion are defined by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the European Food Safety Authority (EFSA). In the US, the ADI levels are regulated by the FDA and are listed in terms of dye intake in mg per 60-kg person (**Table 3**). To achieve an ADI upper limit of Brilliant Blue FCF (ADI: 720 mg/person/day), a 60-kg person would have to inhale 14 kg of the blue e-liquid per day. In order to achieve an ADI value for Allura Red AC (ADI: 420 mg/person/day), one would have to inhale 6 kg of red e-liquid. For Tartrazine (ADI: 300 mg/person/day), the value would be 1 kg of green e-liquid. To reach average per-capita exposure levels listed by FDA (**Table 3**), a person would have to consume 34, 268 and 410 g of blue, red and green e-liquids, respectively (per-capita exposure levels for Brilliant Blue FCF, Allura Red AC and Tartrazine are 1.72, 17.91 and 12.06 mg/60-kg person/day (**Table 3**)).

In addition to the dilemma of inhaling these dyes, food colorants in e-liquids may produce toxic byproducts via thermal degradation. Some of the potential degradants can include dye precursors and aromatic amines that are considered to have carcinogenic potential.³⁷

In vaped samples in the investigation herein, dye desulfonation was observed at a moderate operational power of the e-cigarette (**Table 7** and **Supplementary Information**). The presence of sulfate (SO_4^{2-}) ions in the collected aerosols indicates that sulfur dioxide (SO_2) or/and sulfur trioxide (SO_3) are released from the sulfonated dyes (and oxidized to SO_4^{2-} according to the method described by Makkonen *et al.* for analysis).³⁸ This suggests that the dyes start to degrade at temperatures as low as those needed to form e-liquid aerosols.⁷ The loss of sulfonate groups via thermal destruction in sulfonated dyes has been previously reported.^{39,40} Rehorek showed that the extent of desulfonation is a function of temperature due to the different positions of sulfonic group in the molecules (i.e., ortho, meta or para to the bonds adjoining the aryl rings), with para-sulfonated compounds having the lowest optimal pyrolysis temperature.⁴⁰ Accordingly, the green e-liquid showed the highest level of SO_4^{2-} in the vaporized samples, as the yellow dye, tartrazine, has two para-sulfonyl groups.

In addition, the samples contained relatively high levels of free chloride (Cl^-) (**Table 7** and **Supplementary Information**). This could be derived from the breakdown of sucralose. The presence of sucralose in the e-liquids was confirmed by nuclear magnetic resonance.⁴¹ Cl^- or SO_4^{2-} were not observed in the air samples, nor in the negative (absorption solution) or positive controls (e-liquid samples diluted in the absorption solution).

Table 7. Sulfate and chloride concentration in vaporized e-liquid samples.

	Replicate	Mass concentration [SO_4^{2-}] ($\mu\text{g/g}$) ^{a,b}			Mass concentration [Cl^-] ($\mu\text{g/g}$) ^a		
		1	2	3	1	2	3
Sample	Red	>LOD	>LOD	>LOD	23.38	56.34	36.48
	Blue	>LOD	0.84	1.96	20.5	33.5	32.82
	Green	>LOD	2.72	2.81	17.27	218.51	184.19

^a analyte yield (μg) per g of e-liquid consumed; ^b SO_4^{2-} was calculated back to SO_2

While the in-depth investigation of sucralose decomposition was not an objective of the current study, finding chloride in the absorption solution suggested the fragmentation of sucralose molecules. The loss of hydrogen chloride (HCl) from sucralose is a known initial step in its breakdown, and can ultimately lead to the release and/or formation of potentially toxic by-products such as chloropropanols.⁴² Recent studies demonstrate that sucralose decomposition, including HCl release and the formation of chlorinated derivatives, occurs at relatively mild temperatures.⁴³⁻⁴⁴ The formation of chlorinated compounds from sucralose in the presence of glycerol (one of the two common e-liquid solvents) and metal oxides is well-precedented.^{45,46}

CONCLUSION

The data presented herein suggests that the infusion of e-cigarette formulations with food additives, including synthetic dyes and sweeteners, creates potential health risks since their inhalation toxicity as well as the toxicity of their byproducts is largely unknown. We hope the results of this study will increase awareness and inform decisions about the regulation of e-cigarettes, whether as inhalable foodstuffs, or as nicotine delivery matrices primarily intended for smoking cessation.

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SUPPLEMENTARY INFORMATION

HPLC-DAD chromatograms

Figure S1. Additional chromatograms of dyes standards.
Standards at 40 $\mu\text{g/mL}$ concentrations.

ESI-HRMS spectra of synthetic dye standards

Figure S2. ESI-HRMS spectrum of Allura Red AC Standard.
 $[M]^{2-}$ ion. Theoretical mass: 225.00903. Observed mass: 225.00924. $\Delta m/z$: 0.93 ppm

Figure S3. ESI-HRMS spectrum of Erythrosine Standard.
 $[M]^{2-}$ ion. Theoretical mass: 834.64667. Observed mass: 834.64394. $\Delta m/z$: 3.27 ppm

Figure S4. ESI-HRMS spectrum of Fast Green FCF Standard
 $[M]^{2-}$ ion. Theoretical mass: 381.06823. Observed mass: 381.06713. $\Delta m/z$: 2.89 ppm

Figure S5. ESI-HRMS spectrum of Tartrazine Standard
 $[M]^{3-}$ ion. Theoretical mass: 154.99315. Observed mass: 154.99263. $\Delta m/z$: 3.35 ppm

Figure S6. ESI-HRMS spectrum of Sunset Yellow FCF Standard.
 $[M]^{2-}$ ion. Theoretical mass: 202.99592. Observed mass: 202.99515. $\Delta m/z$: 3.79 ppm

Figure S7. ESI-HRMS spectrum of Brilliant Blue FCF Standard.
 $[M]^{2-}$ ion. Theoretical mass: 373.07077. Observed mass: 373.07067. $\Delta m/z$: 0.27 ppm

ESI-HRMS validation of synthetic dyes in e-liquid samples

Figure S8. ESI-HRMS spectrum of Allura Red AC in Red E-liquid.

Theoretical mass: 225.00903. Observed mass: 225.00829. $\Delta m/z$: 3.29 ppm

Figure S9. ESI-HRMS spectrum of Tartrazine in Green E-liquid.

Theoretical mass: 154.99315. Observed mass: 154.99319. $\Delta m/z$: 0.26 ppm

Figure S10. ESI-HRMS spectrum of Brilliant Blue FCF Dye in Green E-liquid.

Theoretical: 373.07077. Observed: 373.07184. $\Delta m/z$: 2.87 ppm

Figure S11. ESI-HRMS spectrum of Brilliant Blue FCF in Blue E-liquid.
Theoretical mass: 373.07077. Observed mass: 373.07220. $\Delta m/z$: 3.83 ppm

Ion chromatograms of vaporized samples

Figure S12. IC of vaporized Red E-liquid.
Bottom to top: replicates 1 – 3 and the spiked sample.

Figure S13. IC of vaporized Blue E-liquid.
Bottom to top: replicates 1 – 3 and the spiked sample.

Figure S14. IC of vaporized Green E-liquid.
Bottom to top: replicates 1 – 3 and the spiked sample.

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