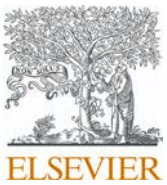
Ecotoxicology and Environmental Safety 267 (2023) 115621



Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Development and validation of a method for preparing heated tobacco product aerosol condensate (HTPAC) for large-scale toxicity data acquisition

Yong-Hyun Kim [a,b,c,*,1], Sung-Hwan Kim [d,1]

[a] Department of Environment & Energy, Jeonbuk National University, 567 Baekje-daero, Deokjin-gu, Jeonju-si, Jeollabuk-do 54896, Republic of Korea
[b] School of Civil, Environmental, Resources and Energy Engineering, Jeonbuk National University, 567 Baekje-daero, Deokjin-gu, Jeonju-si, Jeollabuk-do 54896, Republic of Korea
[c] Soil Environment Research Center, Jeonbuk National University, 567 Baekje-daero, Deokjin-gu, Jeonju-si, Jeollabuk-do 54896, Republic of Korea
[d] Jeonbuk Branch Institute, Korea Institute of Toxicology, Jeongeup-si, Jeollabuk-do 56212, Republic of Korea

ARTICLE INFO

Edited by Dr G Liu

Keywords:
Heated tobacco product (HTP)
Heated tobacco product aerosol condensate (HTPAC)
Volatile organic compound (VOC)
Semi-volatile organic compound (SVOC)
Carbonyl compound

ABSTRACT

A method of preparing heated tobacco product aerosol condensate (HTPAC) was developed to expedite HTP toxicity evaluation, and the effectiveness was assessed. To prepare HTPAC, HTP aerosol was generated and collected using a Cambridge filter (particulate phase) and Dulbecco's phosphate buffered saline (DPBS; gaseous phase). The aerosol collected on the Cambridge filter was extracted using methanol, which was thereafter removed by nitrogen purging. The HTP aerosol residue was mixed with DPBS loaded with the collected HTP vapor, ultimately yielding HTPAC. Nicotine and formaldehyde, key harmful compounds in HTP aerosol, were detected in HTPAC (901 ± 224 and 22.2 ± 3.90 μg stick^{-1}, respectively, comparable to those in HTP aerosol (990–1350 (nicotine) and 2.33–21.9 μg stick^{-1} (formaldehyde)). Propylene glycol and vegetable glycerin, which influence the amount of HTP aerosol, were detected at similar levels in HTPAC and HTP aerosol (propylene glycol = 616 ± 57.1 (HTPAC) and 320–630 μg stick^{-1} (aerosol) and vegetable glycerin = 2418 ± 224 (HTPAC) and 1667–4000 μg stick^{-1} (aerosol)). Known components of HTP aerosol (hydroxyacetone, acetic acid, triacetin, and 2-furanmethanol) were also detected in HTPAC. Consequently, HTPAC offers an effective method for concentrating harmful compounds found in HTP aerosols. This, in turn, facilitates comprehensive toxicity assessments, paving the way for guidelines ensuring the safe utilization of HTP.

1. Introduction

Heated tobacco products (HTPs), emerging as alternatives to conventional cigarettes, have seen continuous development. Diverse HTPs (devices and sticks) have been released due to the high demand and the technological advancements achieved by manufacturers (Akiyama and Sherwood, 2021; Bialous and Glantz, 2018; Jankowski et al., 2019). The launch of these latest HTPs is promoting the influx of new users. The global market value of HTP in 2021 was an impressive $416 billion, and is projected to grow to approximately $800 billion by 2027 (Research And Markets, 2022). The majority of the HTP market share is dominated by Philip Morris International (USA) with IQOS, KT&G (Republic of Korea) with Lil, and British American Tobacco (UK) with Glo (Miller et al., 2022; Seo et al., 2023). IQOS, Lil, and Glo consistently improve user convenience and refine designs by developing HTP devices, thereby increasing the accessibility for smokers. Additionally, they have continued to develop new HTP stick products with a variety of scents to cater to the diverse preferences of smokers. Recently, Ploom Tech from Japan Tobacco Industry (JTI) and PAX Era products by PAX Labs in the US have also gained popularity (Kostygina et al., 2022; Tattan-Birch et al., 2022). As consumers are exposed to an array of HTP devices and sticks from different manufacturers, it is crucial to closely manage and assess the health and safety implications of HTP usage to ensure user safety.

HTP manufacturers assert that HTPs are substantially less harmful than traditional cigarettes. Some studies have found that HTP aerosols

* Corresponding author at: Department of Environment & Energy, Jeonbuk National University, 567 Baekje-daero, Deokjin-gu, Jeonju-si, Jeollabuk-do 54896, Republic of Korea.
E-mail address: ykim84@jbnu.ac.kr (Y.-H. Kim).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.ecoenv.2023.115621
Received 26 June 2023; Received in revised form 17 October 2023; Accepted 19 October 2023
Available online 23 October 2023

contain lower concentrations of various organic compounds (OCs), specifically, volatile organic compounds (VOCs) and semi-volatile organic compounds (SVOCs)), than cigarette smoke and exhibit relatively minor toxic effects (Başaran et al., 2019; Chen-Sankey et al., 2023; Dusautoir et al., 2021; East et al., 2021; Hirano et al., 2020; Kim and An, 2020; Kim et al., 2020; Kopa and Pawliczak, 2020; Zhu et al., 2022). However, the concentration of specific OCs (e.g., nicotine, formaldehyde) in HTP aerosols is similar to or higher than in that in conventional cigarettes (Başaran et al., 2019; Dusautoir et al., 2021; Hirano et al., 2020; Kim and An, 2020; Kim et al., 2020; Kopa and Pawliczak, 2020). Given the lack of clear experimental evidence demonstrating HTP safety, further hazard research on HTPs is necessary.

Inhaling aerosols from HTPs poses health hazards similar to those associated with traditional cigarette smoke. Although HTP aerosols might have reduced levels of toxic compounds compared to conventional cigarette smoke, the degree of human exposure to these chemicals from HTPs can be influenced by user behaviors and device functionality (Camoni et al., 2023; Rabenstein et al., 2023; Zhan et al., 2019). Inhalation of these compounds heightens the risk of respiratory ailments, cardiovascular disorders, and various malignancies (Bhat et al., 2021; Morjaria et al., 2023; Nakama and Tabuchi, 2021; Rodrigo et al., 2021). Specific agents like nitrogen oxides, aldehydes, hydrocyanic acid, acrolein, and ammonia consistently induce irritation, inflammation, and ciliary impairment upon inhalation (Behr and Nowak, 2022). Compounds such as aromatic hydrocarbons, phenols, nitrosamines, and polycyclic aromatic hydrocarbons are identified as carcinogenic (Behr and Nowak, 2022). Indole and carbazole have associations with tumor development (Behr and Nowak, 2022). Compounds such as propylene glycol and vegetable glycerin, common in many electronic nicotine delivery systems (ENDS) including HTPs, might enhance the delivery of toxins to the lungs, amplifying the adverse effects of HTP aerosols (Carll et al., 2022). As with conventional cigarettes, the addictive potential of nicotine in HTP aerosols warrants attention (Lau et al., 2022; Lee et al., 2023). Hence, a comprehensive and timely evaluation of HTP toxicity is essential to clarify the health implications of their use.

HTP hazard assessments involve qualitative and quantitative analyses of hazardous chemicals in HTP aerosols, along with in vivo and in vitro toxicity data derived from exposure to HTP aerosol. The analysis of HTP aerosols varies depending on the type of harmful chemicals they contain (Bansal and Kim, 2016). For VOCs, chromatography is routinely employed for separation, in conjunction with mass spectrometry (MS) for both qualitative and quantitative determinations (Kim and Kim, 2015a; Lim et al., 2023). Volatile components such as formaldehyde undergo derivatization before being isolated using high-performance liquid chromatography (HPLC). Their quantification is subsequently achieved through detectors including UV, fluorescence (FLD), or MS (Kim et al., 2020; Nishimoto-Kusunose et al., 2022). Furthermore, larger molecular weight constituents, such as nitrosoamines, are predominantly examined using HPLC-MS/MS systems (Onoue et al., 2022; Shaik et al., 2022). Trace levels of heavy metals are more conventionally assessed through inductively coupled plasma (ICP)-MS or atomic absorption spectroscopy (AAS) rather than discrete MS systems (Miranda et al., 2012; Szukalska et al., 2021). Recent advancements have seen the deployment of electrochemical sensors for swift detection of less dense harmful chemicals in HTP aerosols, examples being carbon monoxide or hydrogen sulfide (Aranyosi et al., 2022). Consequently, precise evaluation of HTP aerosols demands the discerning choice of an instrumentation system tailored to the specific harmful chemicals under investigation. Prior to analyzing HTP aerosols and evaluating their toxic effects, it is crucial to generate HTP aerosols and obtain samples. Typically, HTP aerosol is generated using a cigarette smoke generator designed for regular cigarettes (Gasparyan et al., 2018; Leigh et al., 2018; Rodrigo et al., 2021), or through manual generation with a gas-tight syringe (Lim et al., 2023; Lim et al., 2022a; Lim et al., 2022b). HTP manufacturers often develop proprietary HTP aerosol generators for limited use in researching the safety of their products (Helen et al., 2018). For hazard evaluation, cells or animals are exposed to HTP aerosol produced by these methods. In other words, HTP hazard data can only be obtained in laboratories equipped with both HTP aerosol generation and toxicity assessment systems. Cigarette smoke generators (able to concurrently expose over 100 laboratory animals to inhalation) are expensive and limited in general use, and the method of generating HTP aerosol by utilizing gas-tight syringes is currently only applied to HTP aerosol analysis (Lim et al., 2022a; Teague et al., 1994). Therefore, securing and supplying a large quantity of HTP aerosol samples would enable the rapid acquisition of substantial HTP safety data.

To efficiently obtain large quantities of HTP aerosol samples, developing HTP aerosol condensate (HTPAC) is a viable approach. A condensate is a concentrated solution resulting from the phase transformation of particulate matter, such as smoke, aerosols, gaseous substances, into liquid form. The liquid state of HTPAC enables the rapid collection of large sample quantities (HTP aerosol is concentrated by directing it through a solvent), as well as easy storage and transportation, making it accessible for various organizations conducting hazard assessments of tobacco products (DeMarini et al., 2008; Montuschi, 2005). Additionally, subjecting test specimens to various concentrations of HTP aerosol does not necessitate the use of specialized equipment like animal exposure chambers and smoke distribution systems. The liquid form of HTPAC simplifies HTP aerosol concentration control and streamlines hazard assessments. Examples of such assessments include evaluating the long-term toxic effects of HTP aerosols, comparing the hazards of HTP aerosols with those of conventional cigarette smoke, and examining additive toxic effects from the combined use of HTPs and conventional cigarettes. These studies necessitate a range of HTP aerosol concentration groups with precise exposure control.

Cigarette smoke condensate (CSC) was primarily derived from conventional cigarette products. Introduced in the 1950 s, CSC was first used to assess the carcinogenic potential of traditional cigarettes (Rubin, 2001; Thun and Burns, 2001). Subsequently, it has served to evaluate the implications of cigarette smoke inhalation on the development of diverse diseases (Kim et al., 2022a; Kim et al., 2022b; Park et al., 2019). Introduced post-2010, the whole cigarette smoke condensate (WCSC) effectively concentrates both particulate and gaseous phases of cigarette smoke (Kim et al., 2022a; Kim et al., 2022b). By 2023, standardized protocols for WCSC production were established (Kim and Kim, 2023). Presently, there is a pressing need for refined research on the effective concentration of deleterious chemicals in cigarette smoke via WCSC and the development of condensates for an array of tobacco products, including e-cigarettes.

It is crucial to rapidly assess the toxicity of HTPs and obtain substantial research data on HTP safety. In this study, we develop and validate a method of standardizing HTPAC to obtain diverse hazard data on HTPs. HTPAC is produced by collecting HTP aerosol using a filter and solvent, extracting the aerosol from the filter, and mixing it with the solvent. Throughout this series of procedures, the loss of harmful chemicals in the aerosol is examined using standard solutions. Subsequently, HTPAC is derived using an HTP device and stick from a leading HTP manufacturer, and the concentrations of major OCs and carbonyl compounds in HTPAC are assessed. The results of the analyses of hazardous chemicals in HTPAC are compared with prior research data on HTP aerosol, and the recovery of these hazardous chemicals in HTPAC is evaluated.

2. Materials and methods

2.1. HTPAC preparation

To expedite research on the hazards of HTP, we established a standardized method for preparing HTPAC. This method was adapted from the standardized procedure our research team previously developed for traditional cigarette smoke condensate (Kim and Kim, 2023).

Recognizing the distinct characteristics of HTP aerosols, notably their concentration and chemical constituents, compared to conventional cigarette smoke, we crafted a tailored standardization protocol for HTPAC. The detailed procedure for HTPAC preparation is presented in Fig. 1.

1. Attach ten sticks to the HTP device and connect the stick tips to the cigarette inlet of the cigarette smoke generator (SG-300, Sibata, Japan).
2. Connect the HTP aerosol outlet of the cigarette smoke generator to the inlet of the filter holder, equipped with a 44 mm Cambridge filter (GE Healthcare, Buckinghamshire, UK).
3. Connect the filter holder outlet to an impinger with a capacity of less than 100 mL, filled with 49.5 mL of Dulbecco's phosphate buffered saline (DPBS).
4. Operate the HTP device and cigarette smoke generator to produce HTP aerosol under HCI regimen conditions (puff duration = 2 s, puff volume = 55 mL, filter vent blocking = 100%, puff number = 8–12, and interpuff interval = 30 s).
5. The HTP aerosol passes through the Cambridge filter, collecting particles, while the HTP vapor is collected by absorption in DPBS.
6. Replace the ten sticks with new ones and repeat steps 4 and 5. Collect HTP aerosol generated from the 80 sticks on one Cambridge filter and in 49.5 mL of DPBS.
7. Place the Cambridge filter with the collected HTP aerosol particles in a conical tube containing 5 mL of methanol. Stir for 1 h at 500 rpm using a vortex mixer (IG-S30MIX, IGene Labserve, India) for particle extraction.
8. Pass nitrogen through the HTP aerosol extract at a rate of 2 L min^{-1} for 3 h using a nitrogen evaporator (NDK200–2 N, MIULAB, China) to remove methanol and concentrate the extract.
9. Add 0.5 mL of dimethyl sulfoxide (DMSO, purity > 99.9%) to the conical tube containing the extract residue. Stir for 5 min at 100 rpm using a vortex mixer.
10. Transfer the DPBS from the impinger, where the HTP vapor was collected, to a 50 mL volumetric flask.
11. Combine the stirred extract residue with the DPBS in the volumetric flask. Complete HTPAC preparation by stirring at 100 rpm for 5 min using a vortex mixer.

2.2. Experimental scheme

The HTPAC preparation method was validated, where the procedures for HTP aerosol sampling and pretreatment were divided into six stages (Exps 1–6), and each stage was verified accordingly (Fig. 1).

Exp 1 assessed the mass of HTP aerosol that could be collected by the Cambridge filter without breakthrough. Exp 2 evaluated the efficiency of methanol extraction for HTP aerosol (SVOCs) collected by the Cambridge filter. Exp 3 assessed the efficiency of DPBS in absorbing HTP vapor (VOCs) (DPBS solvent effect). Exp 4 examined SVOC loss from the extract when methanol, the solvent for HTP aerosol extraction, was removed using nitrogen flow. Exp 5 evaluated the solubility of HTP aerosol in DMSO (DMSO solvent effect). In Exp 6, HTPAC was prepared according to the method described in Section 2.1, and the concentrations of OCs (SVOCs and VOCs) and carbonyl compounds in HTPAC were analyzed and compared to those in HTP aerosol.

HTP aerosol samples from three major HTP manufacturers were used in Exp 1 and Exp 6. Exps 2, 3, 4, and 5 were conducted using working standards and solutions. Exps 2, 4, and 5, which verified the recovery of HTP aerosol (particulate phase), employed working standards and solutions with four SVOCs (propylene glycol, nicotine, triacetin, and vegetable glycerin). In Exp 3, which evaluated the recovery of HTP vapor (gaseous phase), working standards and solutions with four VOCs (n-valeraldehyde, methyl isobutyl ketone, toluene, and n-butyric acid) were utilized. In Exp 6, the SVOCs and carbonyl compounds in HTPAC were quantitatively analyzed using working standards comprising four SVOCs and five carbonyl compounds (formaldehyde, acetaldehyde, acetone, propionaldehyde, and crotonaldehyde), respectively (Table S1). The target SVOCs were selected due to their notable concentrations in HTP aerosols (Kim and An, 2020; Kim et al., 2020). We selected the four VOCs for analysis based on their diverse molecular weights and polarities, as these factors could influence their absorption efficiencies in DPBS. The heating process of the HTP stick leads to the substantial production of the five carbonyl compounds (Kim et al., 2020;

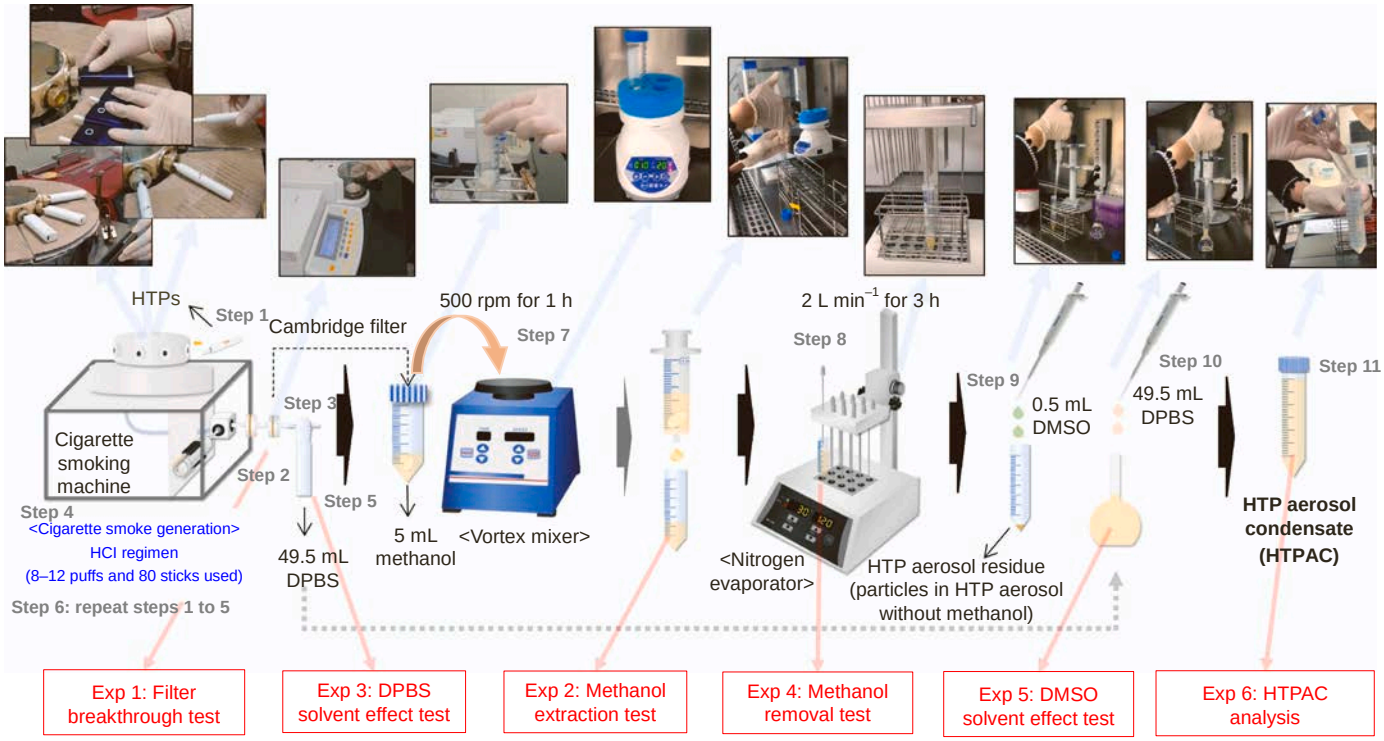


Fig. 1. HTPAC preparation process and validation methods.

Uchiyama et al., 2018).

2.3. Preparation of working standards and solutions

In all HTPAC validation experiments except Exp 1, working standards (for obtaining calibration data) and working solutions (for evaluating the calibration characteristics) containing OCs were employed. These standards and solutions were grouped based on the OC types: Group A (four SVOCs), Group B (four VOCs), and Group C (five carbonyl compounds). Group A was subdivided into three categories with varying concentrations for different experimental purposes: A-1 (Final working standard (WS) concentrations: 14.0 ± 1.45, 27.9 ± 2.90, 55.9 ± 5.80, and 140 ± 14.5 ng μL^{-1}), A-2 (primary and working spiking solution (PSPI and WSPI) concentrations: 2220 ± 219 ng μL^{-1} and 44.4 ± 4.37 ng μL^{-1}), and A-3 (working sample solution (WSAM) concentrations: 7.98 ± 0.83, 39.9 ± 4.14, and 79.8 ± 8.28 ng μL^{-1}). Group B included four Final WS concentrations at levels of 10.6 ± 0.99, 21.2 ± 1.98, 42.5 ± 3.95, and 106 ± 9.88 ng μL^{-1}. Group C featured five Final WS concentration levels of 0.06, 0.12, 0.30, 1.50, and 3.00 ng μL^{-1}. Methanol served as the solvent for all standards and solutions; DPBS and DMSO were used for specific experimental purposes. Details of the methods of preparing the standard samples and solutions, as well as their application in the experiments, are presented in Table S2. The prepared liquid samples were analyzed using instrumental analysis to obtain basic calibration data and detection limits for the target organic compounds (OCs, n = 13) as shown in Table S3.

2.4. Instrumental system

All samples were analyzed using two analytical systems: (1) GC (GC-2010, Shimadzu, Japan) coupled with MS (GCMS-QP2010 ultra, Shimadzu, Japan), and (2) HPLC (Shimadzu, Japan). Samples from Exps 2, 3, 4, and 5 were analyzed by GC-MS, whereas HTPAC samples from Exp 6 were analyzed using both GC-MS and HPLC. The operational conditions for each analytical system are outlined below (see Table S4).

2.4.1. GC-MS system

In the analysis, 1 μL of sample was injected into the GC injector at 250 °C using the auto sampler (AOC-5000, Shimadzu, Japan). The target analytes were then separated on a DB-5MS column (length: 60 m, diameter: 0.25 mm, and film thickness: 0.25 μm; Agilent, USA) using helium (>99.999%) as the carrier gas at a constant flow rate of 2 mL min^{-1}. The GC oven temperature was initially set to 100 °C for 3 min, ramped to 280 °C at 60 °C min^{-1}, and held at this temperature for 9 min, resulting in a total run time of 15 min

The target analytes separated by the GC system were then subsequently detected by the MS system. Both the interface and ion source temperatures were set to 230 °C. The target analytes were quantified in total ion chromatogram mode in the mass range of 30–600 *m/z*. Extracted ion chromatogram mode was then applied to minimize the interfaces by using significant ions identified from the spectrum of each target analyte (Table S1).

2.4.2. HPLC system

HTPAC samples from Exp 6 were injected into the HPLC system using an auto sampler (SIL-20A, Shimadzu, Japan) and pump (LC-20AD, Shimadzu, Japan). Carbonyl compounds in the samples were separated with a Shim Pack GIS-ODS column (length: 250 mm, diameter: 4.6 mm, and particle size: 5 μm; Shimadzu, Japan) using a mobile phase comprising distilled water and acetonitrile (3:6 by volume) at a flow rate of 1.5 mL min^{-1}. The oven temperature was fixed at 30 °C (CTO-20A, Shimadzu, Japan); the total run time for each analysis was 25 min. The separated carbonyl compounds were detected by a UV detector (SPD-20A, Shimadzu, Japan) at a wavelength of 360 nm (Table S4).

2.5. Experimental approaches: Evaluation of the HTPAC preparation method through validation experiments

The HTPAC preparation method underwent validation through six distinct stages, with key parameters for each phase detailed in Table S5.

2.5.1. Evaluation of breakthrough for particle collection in the HTP aerosol using Cambridge filter (Exp 1)

Ten sticks were attached to the HTP device and connected to the cigarette inlet of the smoke generator. The HTP aerosol outlet was linked to the inlet of a filter holder with a Cambridge filter (Front filter), followed by another filter holder with a Cambridge filter (back filter). To generate the HTP aerosol, the HTP device and cigarette smoke generator were operated under HCI/ISO intense regime conditions (puff duration = 2 s, puff volume = 55 mL, filter vent blocking = 100%, puff number = 8–12, and interpuff interval = 30 s); the aerosol was collected sequentially through both filters. After puffs, the sticks were replaced, and the experiment was conducted using 40, 80, 120, and 180 sticks, respectively. The number of HTP sticks used (40, 80, 120, and 180 sticks) was determined based on the breakthrough mass levels of the Cambridge filter for cigarette smoke, ensuring it falls within the range where breakthrough could occur (Kim and Kim, 2023). The Cambridge filters were weighed using a balance (LE225D, Sartorius, USA), and the mass concentration (mg $stick^{-1}$ or μg $stick^{-1}$) of HTP aerosol was calculated to determine the effective collection capacity of the filter for the used HTP sticks. Three types of HTP sticks (sample code: sample A, B, and C) were used in all tests. The three target HTP sticks correspond to the top three products in the South Korean market based on the 2022 market share in terms of volume.

2.5.2. SVOC recovery from Cambridge filter by methanol extraction (Exp 2)

PSPI (40 μL) was spiked onto a Cambridge filter, placed in a 5 mL conical tube, and mixed with 1960 μL of methanol. The tube was vortexed at 500 rpm for 1 h to extract the four SVOCs collected on the filter. The resulting solution, designated as WSPI, was analyzed using a GC-MS system. The efficiency of methanol for extraction of the samples was assessed by comparing the theoretical and analytical (measured) concentrations of WSPI. The 'measured' concentration of WSPI was determined using calibration data obtained from analysis of the Final WS (Group A).

2.5.3. VOC absorption in DPBS (solvent effect) for collection of HTP vapor (Exp 3)

Final WS (Group B) was prepared using methanol and DPBS as solvents, and analyzed by GC-MS. Linear regression analysis was conducted to determine the regression equation relating the analyte mass (pg, injected into the GC-MS) to the corresponding peak area, representing the analytical sensitivity. The absorption efficiency of DPBS for the four VOCs was assessed by comparing the slopes (pg^{-1}) of the regression lines obtained for the two types of Final WS with different solvents.

2.5.4. Methanol removal using nitrogen gas stream (Exp 4)

A 2 mL aliquot of the previously prepared 7 mL of Final WSAM was transferred into vials and analyzed by GC-MS. The remaining 5 mL of Final WSAM was placed in a nitrogen evaporator, where the methanol was removed under nitrogen flowing at 2 L min^{-1} for 3 h (this process is termed nitrogen concentration and involves solvent removal by blowing with nitrogen gas). Subsequently, methanol was added to adjust the final volume back to 5 mL, and the sample was analyzed by GC-MS. The analytical sensitivity (slope, pg^{-1}, peak areas against analyte mass injected into the GC-MS) obtained from the final WSAM before and after nitrogen concentration was compared.

2.5.5. DMSO solvency (solvent effect) for ensuring homogeneous mixing of HTPAC components (Exp 5)

The final WS (Group A) was prepared using methanol and DMSO as solvents and subsequently analyzed by GC-MS. Linear regression analysis of the analytical mass (pg, injected into the GC-MS) and the peak area was performed, and the slope (pg^{-1}) of the regression line was calculated. The solubility of the four SVOCs in DMSO was evaluated by comparing the slopes for the two types of Final WS prepared with different solvents (methanol and DMSO).

2.5.6. Qualitative and quantitative analyses of SVOCs and carbonyl compounds in HTPAC (Exp 6)

HTPAC was obtained from three types of HTP according to the preparation method described in Section 2.1 (samples A, B, and C). These samples were analyzed using GC-MS and HPLC-UV. The concentrations of propylene glycol, nicotine, triacetin, vegetable glycerin, formaldehyde, acetaldehyde, acetone, propionaldehyde, and crotonaldehyde in HTPAC were quantitatively evaluated.

3. Results and discussion

3.1. Evaluation of breakthrough for particle collection from HTP aerosol (Exp 1) using Cambridge filter

In Exp 1, the maximum mass of HTP aerosol that can be collected without breakthrough using a Cambridge filter was assessed. Additionally, the mass of HTP aerosol was divided by the number of HTP sticks employed for aerosol generation, and the HTP aerosol concentration (mg $stick^{-1}$ or μg $stick^{-1}$) was calculated as follows: Concentration (mg $stick^{-1}$ or μg $stick^{-1}$) = Mass of HTP aerosol collected (mg or μg) on Cambridge filter / Number of HTP sticks used.

First, we examined whether the concentration of HTP aerosol collected by the front filter varied depending on the number of HTP sticks used for aerosol generation. The average mass of HTP aerosol generated by 40 HTP sticks was 389 ± 68.0 mg, corresponding to an average concentration of 9.73 ± 1.70 mg $stick^{-1}$ (Fig. 2). As the number of HTP sticks used increased, the concentration of HTP aerosol decreased. The concentration of HTP aerosol generated using 80 and 120 HTP sticks was 9.07 ± 1.80 mg $stick^{-1}$ and 8.81 ± 1.08 mg $stick^{-1}$, respectively, corresponding to 90% of the concentration produced with 40 HTP sticks. However, when HTP aerosol was generated using 180 HTP sticks, the aerosol concentration significantly decreased to an average of 7.79 ± 0.60 mg $stick^{-1}$, which is about 80% of the

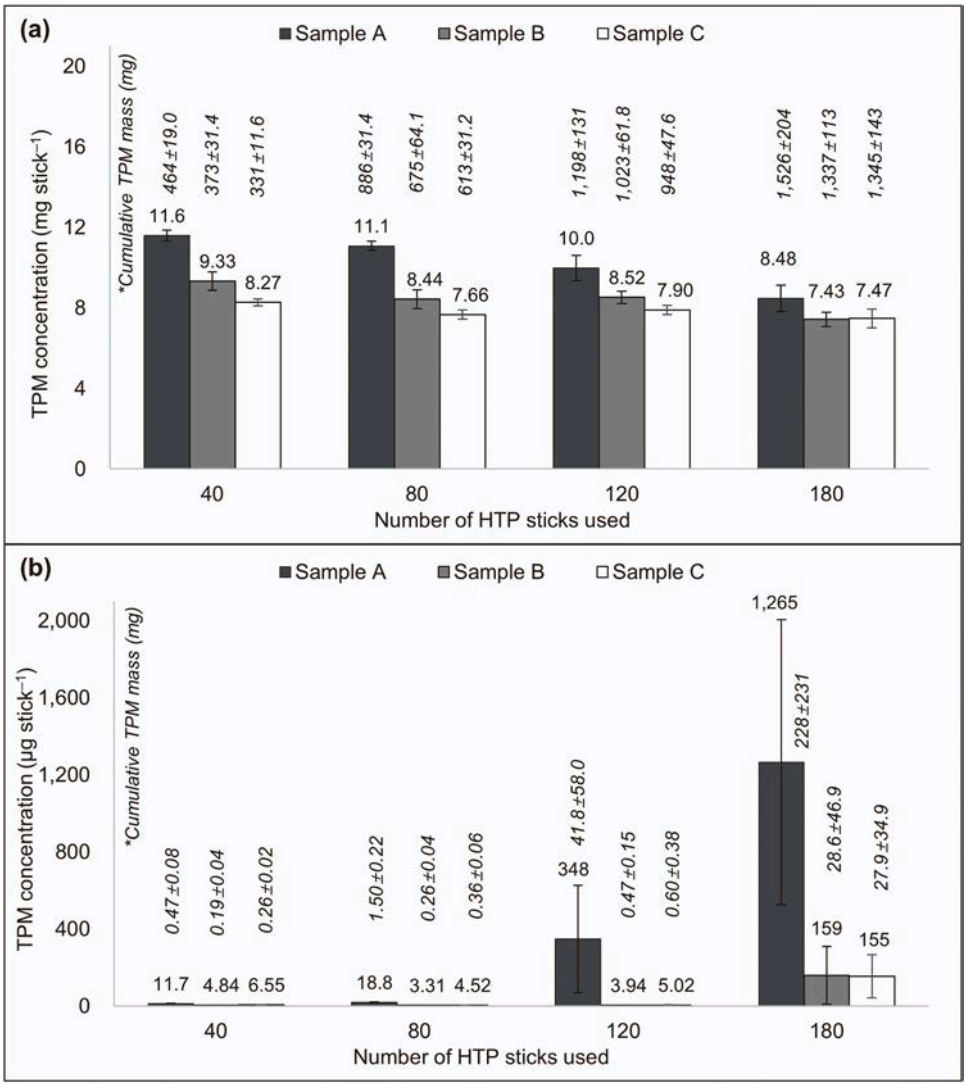


Fig. 2. Capacity of Cambridge filter for collecting TPM in HTP aerosol (Exp 1): (a) front filter and (b) back filter.

concentration generated with 40 HTP sticks. In other words, when HTP aerosol was generated using 180 HTP sticks, a single Cambridge filter was ineffective for collecting a representative level of aerosol.

Comparison of the HTP aerosol concentrations collected by the front filter for the different sample codes showed that the concentrations decreased in the following order: sample A (10.3 ± 1.38 mg stick^{-1}), followed by sample B (8.43 ± 0.78 mg stick^{-1}), and finally sample C (7.83 ± 0.34 mg stick^{-1}). The decrease in the HTP aerosol concentration with an increase in the number of sticks used was most pronounced for sample A, which had the highest concentration of HTP aerosol (–22.9 μg stick^{-1} and R^2 = 0.9826), and was least noticeable for sample C, which had the lowest concentration of HTP aerosol (–4.7 μg stick^{-1} and R^2 = 0.6625). In the case of Sample C, the concentration of HTP aerosol generated using 40 sticks and 180 sticks was 8.27 ± 0.29 mg stick^{-1} and 7.47 ± 0.79 mg stick^{-1}, respectively, with a difference of less than 10%. In other words, based solely on the mass and concentration of HTP aerosol collected with the front filter, generating the aerosol using 180 sticks and collecting it using the Cambridge filter was effective in the case of sample C.

The average mass of the HTP aerosol collected by the back filter was 0.51 ± 0.50 mg for up to 80 HTP sticks, which is relatively insignificant. This corresponds to approximately 5% of the average mass of the aerosol collected by the front filter. In other words, more than 95% of the HTP aerosol generated with 80 HTP sticks could be collected with the front filter (613–886 mg), with a breakthrough of 5% or less (0.19–1.5 mg). Consequently, it was confirmed that up to 886 mg of aerosol can be effectively collected with a single Cambridge filter. When HTP aerosol was generated using 120 HTP sticks and subsequently collected using the back filter for aerosol that had passed through the front filter, the mass of HTP aerosol in sample A was 41.8 ± 58.0 mg, which is approximately 30 times higher than that collected from 80 HTP sticks. On the other hand, the masses of aerosol collected by the back filter in samples B and C remained small, at 0.47 ± 0.15 mg and 0.60 ± 0.38 mg, respectively. However, when HTP aerosol was generated using 180 HTP sticks, the mass of HTP aerosol collected by the back filter increased rapidly, regardless of the sample code (228 ± 231 mg for sample A, 28.6 ± 46.9 mg for sample B, and 27.9 ± 34.9 mg for sample C).

Comprehensive assessment of the mass of HTP aerosol collected using both the front and back filters indicated that up to approximately 886 mg of HTP aerosol, generated with 80 HTP sticks, can be effectively collected per Cambridge filter. Although the amount of HTP aerosol collected from 120 HTP sticks using the front filter remained at a representative level (with a 10% reduction in the HTP aerosol concentration compared to that for 40 HTP sticks), breakthrough was observed for sample A at a concentration level of 0.348 mg stick^{-1} in the back filter. For each HTP stick, sample A exhibited a higher puff count of 12, in contrast to 9 and 8 puffs for samples B and C, respectively. This variation in puff counts is thought to impact the breakthrough of HTP aerosol through the Cambridge filter. Table 1 presents images of the Cambridge filters (front and back filters) used for HTP aerosol sampling.

3.2. SVOC recovery from Cambridge filter via methanol extraction (Exp 2)

In Exp 2, the efficiency (%) of methanol for extraction of the SVOCs collected by the Cambridge filter was evaluated. The methanol extraction efficiency (%) was calculated by assessing the SVOCs recovered from the filter after extraction with 5 mL of methanol. Specifically, the methanol extraction efficiency (%) was determined by comparing the concentration of WSPI (including four SVOCs: propylene glycol, nicotine, triacetin, and vegetable glycerin), which was spiked and subsequently extracted from the Cambridge filter using methanol, with the theoretical concentration (Fig. 3). Methanol extraction efficiency (%) = (Theoretical concentration (ng μL^{-1}) – Analytical concentration (ng

Table 1
Representative image of HTP aerosol captured by Cambridge filter.

Number of HTP sticks used	Sample code					
	Sample A		Sample B		Sample C	
	Front filter	Back filter	Front filter	Back filter	Front filter	Back filter
40						
80						
120						
180						

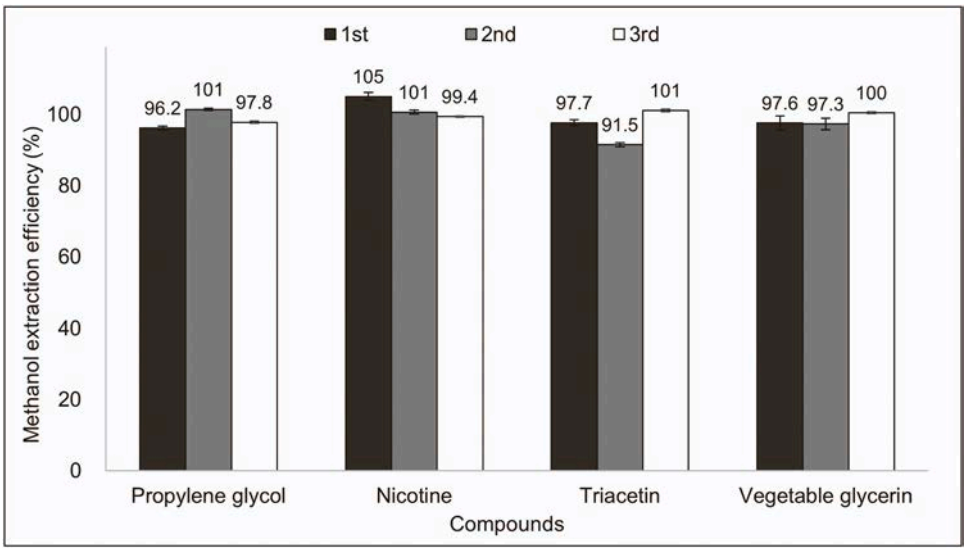


Fig. 3. Assessment of methanol extraction efficiency for SVOCs in HTP aerosol (Exp 2).

μL^{-1})) / Theoretical concentration (ng μL^{-1}) x 100 (%).

Three replicate methanol extraction efficiency evaluations consistently produced excellent agreement, with values exceeding 90% for all SVOC types. The average methanol extraction efficiency for nicotine was 102 ± 2.81%, and it is inferred that 100% recovery by extraction from the Cambridge filter using methanol is feasible, taking into account the variation in the analytical concentration. The methanol extraction efficiencies for propylene glycol and vegetable glycerin were 98.5 ± 2.43% and 98.5 ± 2.72%, respectively. Similar to nicotine, considering the variation in the analytical concentration, it is possible to achieve near 100% recovery of propylene glycol and vegetable glycerin through methanol extraction. The methanol extraction efficiency for triacetin was 91.5% in the second evaluation, but the average efficiency was 96.8 ± 4.33%, indicating a loss of less than 5% when extracted from the Cambridge filter using methanol. Based on the results of Exp 2, it can be concluded that the recovery of SVOCs collected using the Cambridge filter is, on average, greater than 95% when extracted with 5 mL of methanol.

3.3. VOC absorption in DPBS (solvent effect) for collection of HTP vapor (Exp 3)

In Exp 3, the efficiency of DPBS for absorption of the VOCs present in HTP vapor was evaluated in comparison to that of methanol. Because HTP vapor cannot be collected using a Cambridge filter, absorption in a solvent is necessary. Methanol, for instance, can effectively absorb and collect highly volatile VOCs (An et al., 2019; Kim et al., 2013; Sun et al., 2023). However, when evaluating the toxicity of HTPAC by injection into cells and experimental animals, the use solvents with toxic effects exceeding those of HTP emissions (aerosol and vapor) is undesirable. For this reason, non-toxic DPBS was selected as the solvent for absorbing HTP vapor, where the efficiency of DPBS for the absorption and collection of the VOCs was compared with that of methanol. The final WS containing four types of VOCs (n-valeraldehyde, methyl isobutyl ketone, toluene, and n-butyric acid) was prepared using DPBS and methanol as solvents, respectively. The samples were analyzed, and the sensitivity (slope, pg^{-1}) of the analysis for detection of the VOCs based on the type of solvent was compared as follows (Fig. 4). Percentage difference (%) = (Slope of VOCs in methanol (pg^{-1}) – Slope of VOCs in DPBS (pg^{-1})) / Slope of VOCs in methanol (pg^{-1}) x 100 (%).

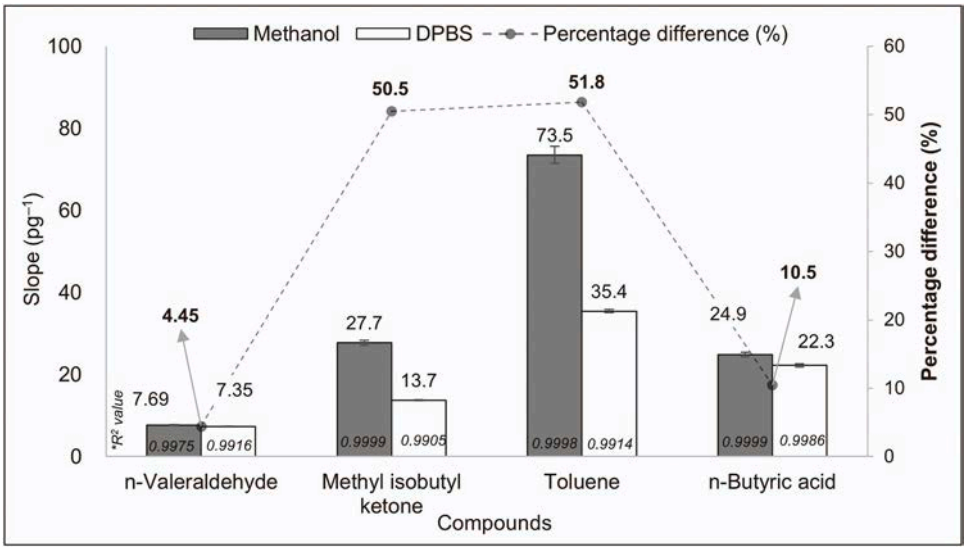


Fig. 4. Evaluation of efficiency of DPBS for absorption of VOCs in HTP vapor (Exp 3).

The slopes for the absorption of n-valeraldehyde were remarkably similar, 7.69 pg^{-1} and 7.35 pg^{-1} in methanol and DPBS solvents, respectively (percentage difference < 5%). n-Butyric acid also exhibited a minimal difference, with a discrepancy of approximately 10% in the value of the slope, attributable to the distinction between methanol and DPBS solvents. However, the slopes for the absorption of methyl isobutyl ketone and toluene in methanol were notably higher compared to that in DPBS (percentage difference = 50.5–51.8%). Toluene is non-polar and does not readily dissolve in DPBS (An et al., 2019; Jafvert and Kulkarni, 2008; Yang et al., 1997). The solubility of methyl isobutyl ketone in water is approximately 14–20 g L^{-1} at 20 °C. It is expected that compared to toluene, isobutyl ketone will be more effective for DPBS absorption and collection (Kawai et al., 2003; Wang et al., 2017). However, the discrepancy in the slope for the absorption of methyl isobutyl ketone in methanol versus DPBS was substantial (~50%). This may be attributed to the relative volatility of methyl isobutyl ketone (vapor pressure = 16 mm Hg at 20 °C) (NIOSH, 2019).

The linearity of the calibration curve obtained through analysis of the Final WS was consistently high (0.99 or greater), regardless of the type of solvent. The concentration of some VOCs remained stable after sampling by absorption in the solvent, although relatively low absorption in DPBS solvent was anticipated. Nonetheless, when HTP vapor is absorbed by DPBS, the limitation for VOCs with relatively low absorption sampling efficiency should be considered (An et al., 2019).

3.4. Methanol removal using nitrogen gas stream (Exp 4)

In the process of obtaining HTPAC, the initial step involves extracting SVOCs from the Cambridge filter after the collection of HTP aerosol, by using methanol as a solvent. However, due to the toxicity of methanol, it must be removed before using HTPAC in cell and animal experiments. In Exp 4, WSAM containing four SVOCs (propylene glycol, nicotine, triacetin, and vegetable glycerin) was prepared using methanol as the solvent. The WSAM was analyzed before and after methanol removal (by applying a nitrogen gas stream), and the slopes for SVOC acquired before and after the removal of methanol were compared (Fig. 5). Percentage difference (%) = (Slope of SVOCs before evaporation (pg^{-1}) – Slope of SVOCs after evaporation (pg^{-1})) / Slope of SVOCs before evaporation (pg^{-1}) x 100 (%).

For all four types of SVOCs, the slope decreased following methanol evaporation; however, the reduction was minimal, within 5%. The difference in the slope before and after methanol evaporation was the smallest (2%) for vegetable glycerin and the largest (6.96%) for triacetin. Nonetheless, the reproducibility (relative standard deviation, RSD), determined through repeated analysis, was 6.17 ± 1.07% and 6.85 ± 0.67% before and after methanol evaporation, respectively, indicating that the degree of SVOC loss due to methanol evaporation did not reach a significant level. In other words, even when methanol was removed from the liquid SVOC sample by evaporation under nitrogen gas at a flow rate of 2 L min^{-1} for 3 h, the loss of the four types of SVOCs with relatively low volatility did not appear to be significant (vapor pressure = 0.002 (triacetin)–0.12 (propylene glycol) mm Hg at 25 °C) (Becker et al., 2019; Martin and Murphy, 2000; Pankow et al., 2004; Quinn Jr and Ziolkowski Jr, 2015). The linearity of the calibration curve obtained through WSAM analysis was excellent, with values above 0.99 for all four SVOCs both before and after methanol evaporation.

3.5. Evaluation of DMSO solvency (solvent effect) to ensure homogeneous mixing of HTPAC components (Exp 5)

The HTP aerosol was collected using a Cambridge filter, followed by methanol extraction, and nitrogen gas was introduced to remove methanol, yielding the HTP aerosol residue. The HTP aerosol residue was then combined with DPBS containing the absorbed HTP vapor, resulting in HTPAC containing both HTP aerosol and vapor components. When mixing HTP aerosol residue with polar DPBS, non-polar OCs in the HTP aerosol residue may not mix well with DPBS, potentially hindering stable analysis. To address this issue, DMSO was added and mixed with the HTP aerosol residue before combining it with DPBS. DMSO is a versatile solvent capable of dissolving both polar and non-polar chemical components (AlOkda and Van Raamsdonk, 2022). Furthermore, DMSO is known to be less toxic than commonly used solvents such as methanol, hexane, and isopropyl alcohol (Belson and Morgan, 2004; Brayton, 1986; Logsdon and Loke, 2000; Ono et al., 1981). Therefore, in this study, DMSO was employed as a surfactant to facilitate the mixing of HTP aerosol residue and DPBS. However, because DMSO may exert biological toxicity depending on its concentration, it was used only after final dilution to a concentration level below 1%, which minimizes the toxic effects of DMSO (Brayton, 1986; Hoyberghs et al., 2021).

In Exp 5, the efficacy of DMSO as a surfactant for mixing HTP aerosol residue with DPBS was assessed. Final WS containing four SVOCs (propylene glycol, nicotine, triacetin, and vegetable glycerin) was prepared using both methanol and DMSO (as solvents), and subsequently analyzed. The solvent effect of DMSO was evaluated by comparing the slope (pg^{-1}) for the absorption of the SVOCs obtained from the final WS analysis with the slope obtained using methanol (Fig. 6). Percentage

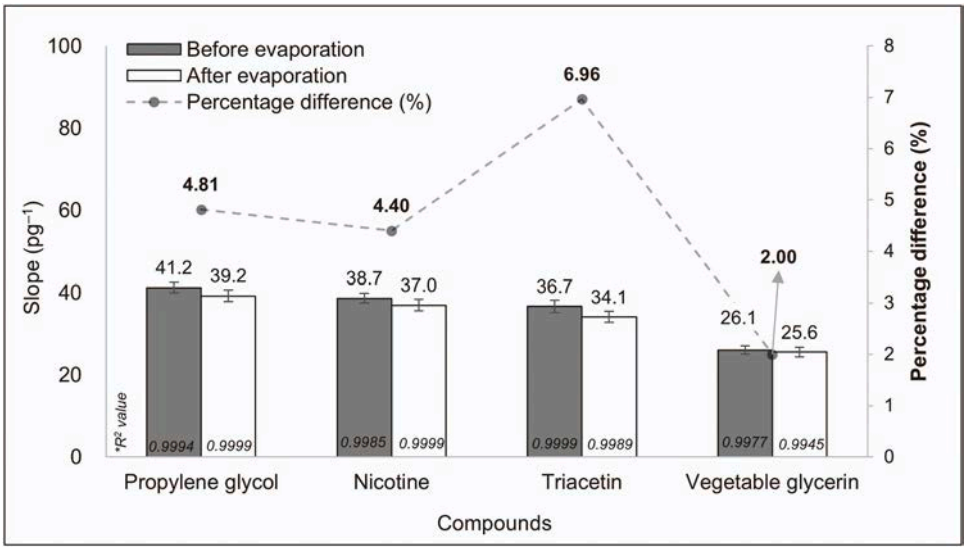


Fig. 5. Evaluation of SVOC loss due to methanol evaporation (Exp 4).

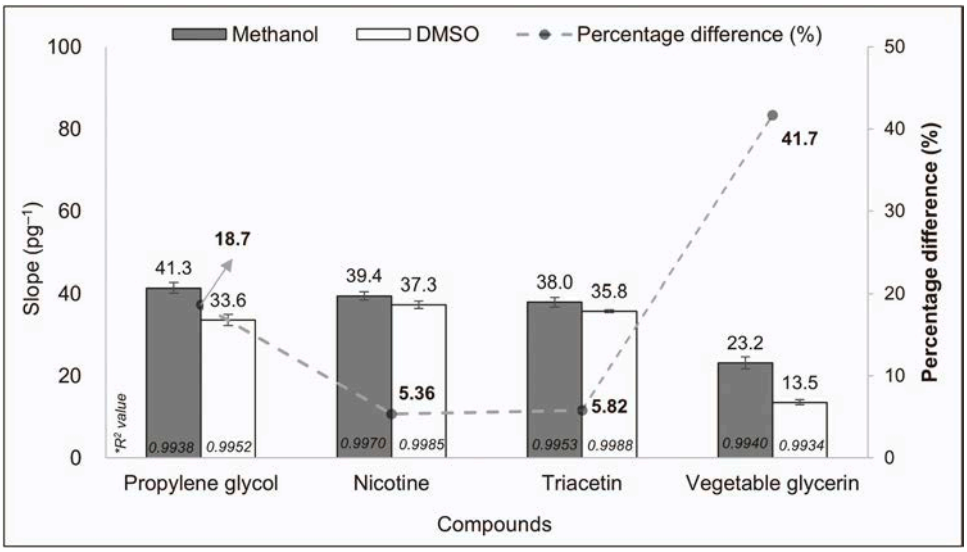


Fig. 6. Evaluation of DMSO solvency (solvent effect) for SVOCs (Exp 5).

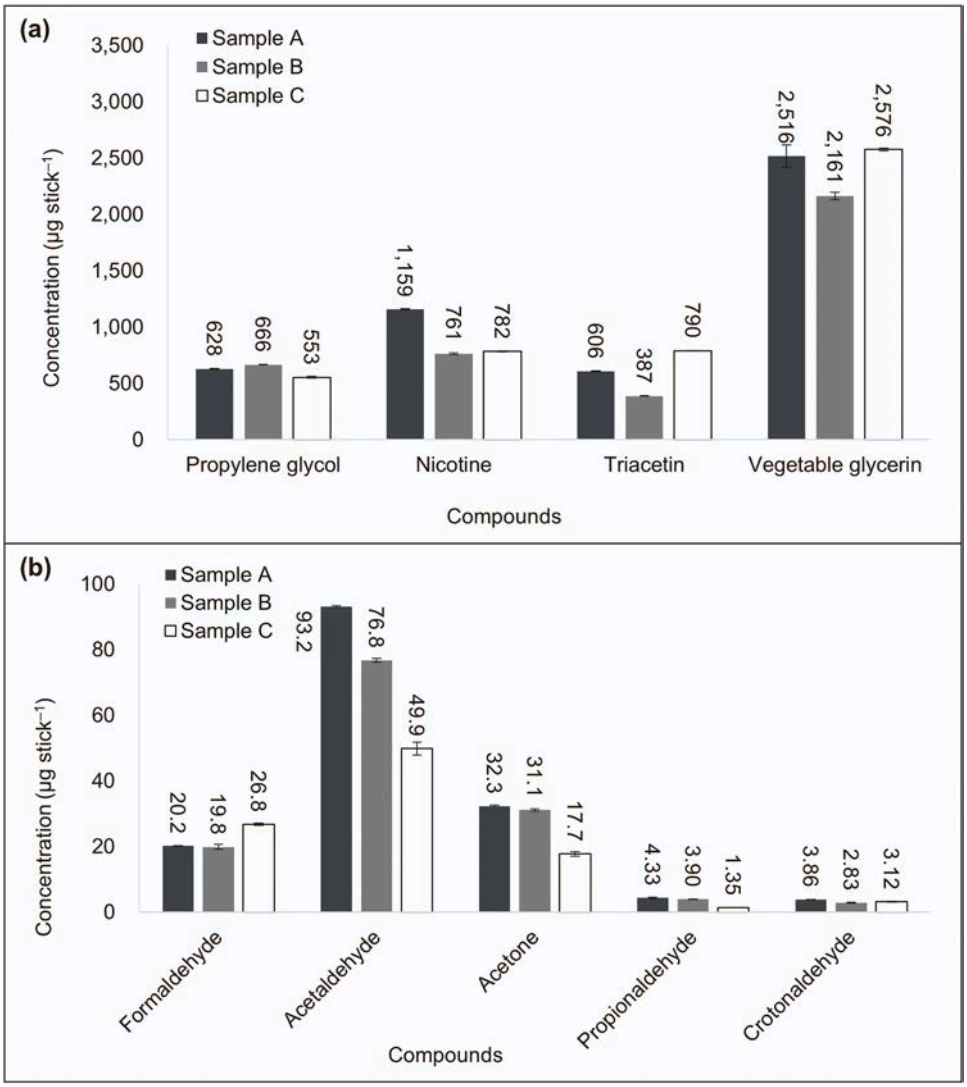


Fig. 7. Concentration of components in HTPAC (Exp 6): (a) four SVOCs and (b) five carbonyl compounds.

difference (%) = (Slope of SVOCs in methanol (pg^{-1}) – Slope of SVOCs in DMSO (pg^{-1})) / Slope of SVOCs in methanol (pg^{-1}) x 100 (%).

The slopes for absorption of the SVOCs in DMSO were consistently smaller than for absorption in methanol. The differences in the slope for the absorption of SVOC is methanol versus DMSO were minimal, at approximately 5% for nicotine and triacetin, but relatively larger at 18.7% and 41.7% for propylene glycol and vegetable glycerin, respectively. In other words, when propylene glycol and vegetable glycerin are dissolved in DMSO solvent, the absolute sensitivity of the analysis appears to be lower than that in methanol solvent. However, the linearity of the SVOC calibration curve obtained through analysis of the final WS was excellent, with values above 0.99 for all SVOCs, irrespective of the solvent. This suggests that the GC-MS system employed in this study had lower analytical sensitivity for the SVOCs in the final WS prepared with DMSO solvent, but maintained good analytical reproducibility. Consequently, the solvent effect of DMSO should be considered in the quantitative evaluation of SVOCs in HTPAC.

3.6. Qualitative and quantitative analyses of SVOCs and carbonyl compounds in HTPAC

Three types of HTPAC (sample A, B, and C) were prepared using the method described in Section 2.1. Four SVOCs (propylene glycol, nicotine, triacetin, and vegetable glycerin) and five carbonyl compounds (formaldehyde, acetaldehyde, acetone, propionaldehyde, and crotonaldehyde) in the HTPAC were analyzed using GC-MS and HPLC-UV, respectively, and the concentrations were calculated (Fig. 7 and Fig. 8). The recovery of the components in HTPAC was evaluated by comparing the concentrations of the SVOCs and carbonyl compounds to those in HTP aerosol (Fig. 9).

The concentrations of propylene glycol, nicotine, triacetin, and vegetable glycerin in HTPAC were 616 ± 57.1, 901 ± 224, 594 ± 202, and 2418 ± 224 μg $stick^{-1}$, respectively. Vegetable glycerin had the highest concentration in all samples, exceeding 2000 μg $stick^{-1}$ and accounting for 21.7–29.6% of the HTP aerosol concentration by mass. The other three SVOCs constituted 4.26 (sample B, triacetin)–9.99% (sample A, nicotine) of the HTP aerosol concentration by mass.

The concentrations of propylene glycol and vegetable glycerin were similar across the sample types (RSD = each 9.28%). In contrast, nicotine and triacetin exhibited larger concentration differences depending on the sample types (RSD = 24.8% for nicotine and 34.0% for triacetin). The highest concentration of nicotine (1159 ± 9.48 μg $stick^{-1}$) was detected in sample A, while triacetin peaked at 790 ± 2.71 μg $stick^{-1}$ in sample C.

Fig. 9 displays results from previous studies that generated and analyzed aerosols using the same HTP type as sample A in this study (Cancelada et al., 2019; Farsalinos et al., 2018; Li et al., 2019; Matsouki et al., 2021; Uchiyama et al., 2018). All studies in Fig. 9 generated HTP

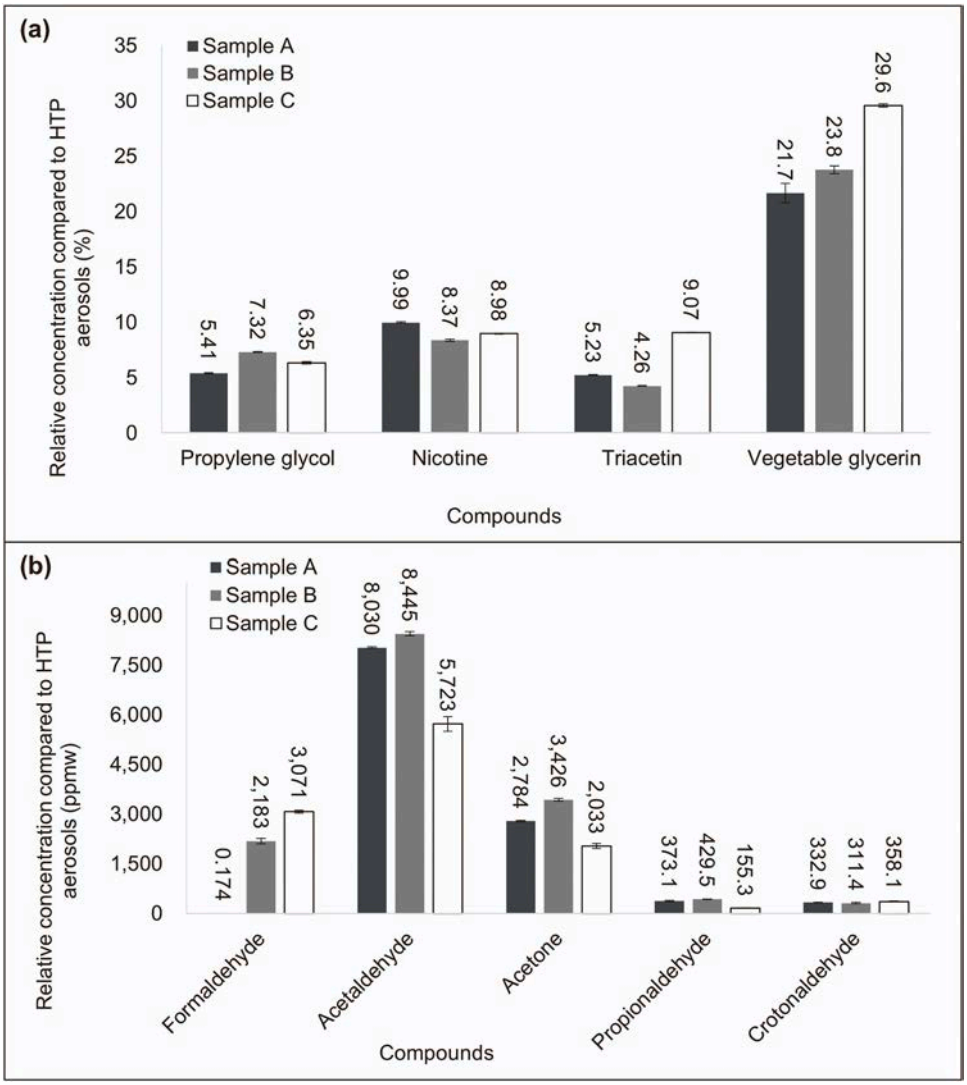


Fig. 8. Relative concentrations of components in HTPAC compared to mass concentrations in HTP aerosol (Exp 6): (a) four SVOCs and (b) five carbonyl compounds.

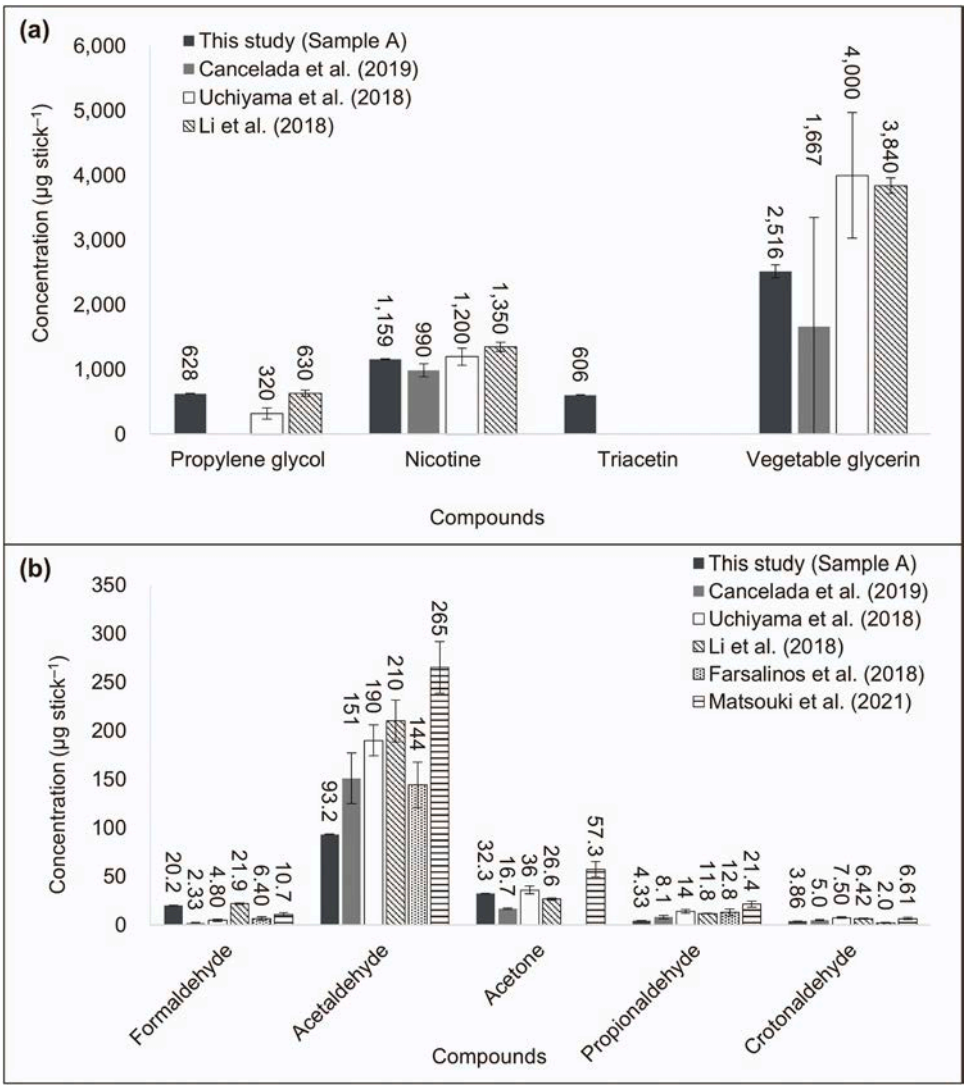


Fig. 9. Comparison of concentrations of major components in HTPAC and HTP aerosol: (a) four SVOCs and (b) five carbonyl compounds.

aerosols under the same HCI regimen conditions with 12 puffs per stick. In previous studies, the nicotine concentrations in the HTP aerosols ranged from 990 to 1350 μg stick^{-1}, similar to the HTPAC nicotine concentration of 1159 ± 9.48 μg stick^{-1} in sample A. The concentration of propylene glycol in the HTPAC (628 ± 8.48 μg stick^{-1}) and HTP aerosols (320–630 μg stick^{-1}) was also comparable. The concentration of vegetable glycerin varied widely in previous studies (1667–4000 μg stick^{-1}), with some reporting RSD values exceeding 100%. The concentration of vegetable glycerin in Sample A was 2516 ± 174 μg stick^{-1}, comparable to that in prior studies, and had relatively better reproducibility (6.93%) based on the RSD. Overall, the concentrations of the major SVOCs in HTPAC closely matched the SVOC levels in HTP aerosols reported in previous research.

The concentration of carbonyl compounds in HTPAC were as follows: acetaldehyde (73.3 ± 21.9 μg stick^{-1}), acetone (27.1 ± 8.11 μg stick^{-1}), formaldehyde (22.2 ± 3.90 μg stick^{-1}), crotonaldehyde (3.27 ± 0.53 μg stick^{-1}), and propionaldehyde (3.20 ± 1.61 μg stick^{-1}), listed in descending order. The acetaldehyde concentration was approximately three times higher than the acetone and formaldehyde concentrations and approximately twenty times higher than the concentrations of crotonaldehyde and propionaldehyde. Therefore, among the analyzed carbonyl compounds, acetaldehyde had the highest concentration in HTPAC.

The concentration of carbonyl compounds in HTPAC varied from 16.3% to 50.4% depending on the sample type. Propionaldehyde had the lowest concentration among the carbonyl compounds, with the largest variation among the samples (RSD = 50.4%). Overall, the concentration of carbonyl compounds in HTPAC exhibited greater differences across the sample types compared to the four target SVOCs. Based on the RSD, the concentration differences for the five carbonyl compounds and four SVOCs were 28.8 ± 13.7% and 19.3 ± 12.2%, respectively.

The concentration differences of the carbonyl compounds in the HTPAC and HTP aerosols varied depending on the compound type. The formaldehyde concentration in HTPAC was 20.2 ± 0.07 μg stick^{-1}, which is approximately twice as high as the average formaldehyde concentration in HTP aerosol (9.23 ± 7.71 μg stick^{-1}). Li et al. (2019) reported a slightly higher formaldehyde concentration in HTP aerosol (21.9 μg stick^{-1}) compared to that in HTPAC. Thus, the formaldehyde concentrations in HTPAC aerosol were either similar to or slightly higher than those in HTP aerosol. However, the acetaldehyde concentration in HTPAC (93.2 ± 0.43 μg stick^{-1}) was ~50% lower than the average concentration in HTP aerosol (192 ± 49.1 μg stick^{-1}). Considering the broad range of acetaldehyde concentrations in HTP aerosol (144–265 μg stick^{-1}), lower concentrations can be detected. The concentrations of acetone in HTPAC (32.3 ± 0.49 μg stick^{-1}) and HTP aerosol (34.2 ± 17.3 μg stick^{-1}) were similar. Additionally, the concentrations of propionaldehyde and crotonaldehyde in HTPAC were 31.8% and 70.2%

of the concentrations in HTP aerosol, respectively. In previous research, even when producing HTP aerosols under consistent conditions with the same HTP, the concentrations of carbonyl compounds exhibited variations, from a 1.84-fold difference (for acetaldehyde) to a 9.40-fold difference (for formaldehyde). The formation of carbonyl compounds results from heating the stick, and the resultant concentrations can markedly differ based on the heating temperature (Kim et al., 2020; Uchiyama et al., 2018). Given these factors, it's plausible to observe

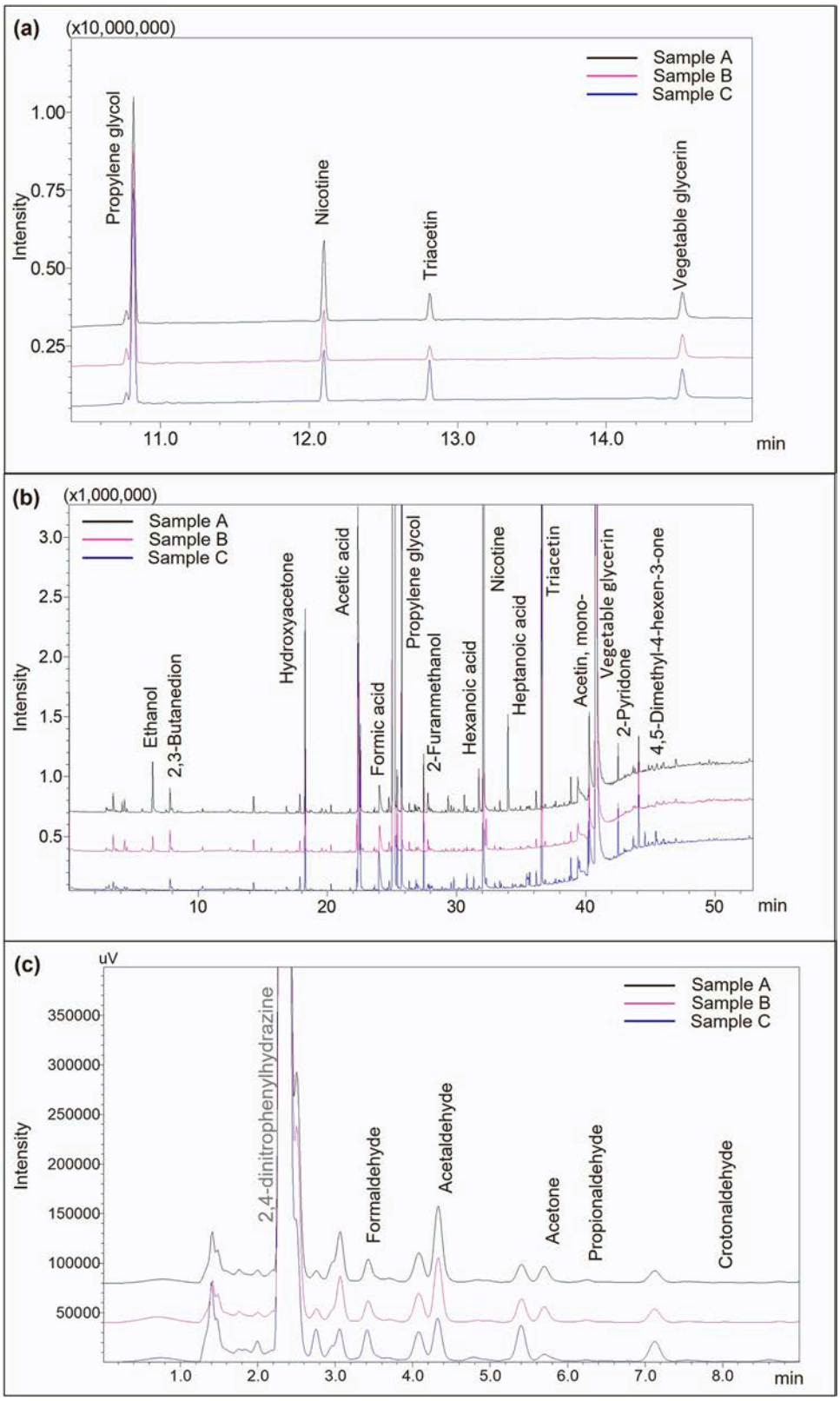


Fig. 10. Chromatograms of VOCs, SVOCs, and carbonyl compounds in HTPAC: (a) 50-fold diluted HTPAC (GC-MS with a DB column), (b) undiluted HTPAC (GC-MS with a wax column), and (c) 100-fold diluted HTPAC (HPLC).

significant variations in the concentration of carbonyl constituents. The carbonyl compound concentrations identified in HTPAC align with the concentration disparities noted across various HTP aerosols.

The HTPAC components, apart from the four target SVOCs and five carbonyl compounds, were qualitatively analyzed (Fig. 10). The chromatogram in Fig. 10(b) mainly presents components with large peak areas. Various components, including nicotine, propylene glycol, and vegetable glycerin, were detected in HTPAC. Among these, hydroxyacetone, acetic acid, triacetin, and 2-furanmethanol are known to be primarily found in HTP aerosol (Crosswhite et al., 2021; Helen et al., 2018; Meehan-Atrash et al., 2019; Nakama and Tabuchi, 2021). This confirms that the components in HTPAC largely match those in HTP aerosol.

Overall, the concentrations of SVOCs and carbonyl compounds in HTPAC were similar to those in HTP aerosol. Some components in HTPAC had slightly lower concentrations, likely due to the solvent effect. However, after adjusting for the reduced sensitivity of the analytical instruments caused by the solvent effect, these concentrations are considered comparable to those in HTP aerosol (An et al., 2019; Kim et al., 2013; Kim and Kim, 2015b). Furthermore, the concentrations of the components in HTP aerosol varied by ~2–5 times across previous studies, indicating that even when the aerosol is generated under the same conditions, the concentrations differ depending on the specific components. Given the concentration variation among prior studies, HTPAC obtained in this study can be considered a suitable representation of HTP aerosol components.

Fig. 10. (Continued) Chromatograms of VOCs, SVOCs, and carbonyl compounds in HTPAC: (a) 50-fold diluted HTPAC (GC-MS with a DB column), (b) undiluted HTPAC (GC-MS with a wax column), and (c) 100-fold diluted HTPAC (HPLC).

4. Conclusions

In this study, we developed HTPAC to quickly obtain safety evaluation data for various HTP types and assessed its effectiveness. HTP aerosol was generated using 80 HTP sticks, where the particulate phase was collected using a Cambridge filter and the gas phase (vapor) by DPBS. In a single experiment, 50 mL of HTPAC was obtained at a concentration level of approximately 10 mg mL^{-1} based on HTP aerosol. During preparation of the HTPAC sample, the SVOCs collected with the Cambridge filter were extracted with methanol, achieving an extraction efficiency of over 90% for all target SVOCs. Methanol was removed by passing nitrogen into the SVOC extract, resulting in minimal SVOC loss (within 7%). However, the analytical sensitivity for some SVOCs was ~50% lower in DPBS and DMSO solvents. For HTPAC preparation, considering the solvent effects of DPBS, which absorbs HTP vapor, and DMSO, which acts as a surfactant for mixing HTP aerosol residue and DPBS, it is necessary to calculate the concentrations of certain components in HTPAC. The concentrations of nicotine, propylene glycol, vegetable glycerin, formaldehyde, acetone, and crotonaldehyde in HTPAC were highly similar to those in HTP aerosol. Additionally, hydroxyacetone, acetic acid, triacetin, and 2-furanmethanol, known to be detected in HTP aerosol, were also found in HTPAC. This suggests that HTP aerosol was effectively concentrated to give HTPAC.

In analyzing the results of this study, the constituent concentrations within both the HTPAC and HTP aerosol demonstrated remarkable similarity. Although the concentrations of particular components, such as acetaldehyde, in the HTPAC were roughly half of those observed in the HTP aerosol, these discrepancies fall within the anticipated range given the inherent variations in component concentrations within HTP aerosol. One limitation of the current study is its primary emphasis on the qualitative assessment of HTPAC concerning major chemical components. Subsequent studies should delve into the qualitative evaluation of other chemical constituents in HTP aerosol not explored in the present research. Additionally, research is needed to optimize the amalgamation of particulate and gaseous components of HTP, gathered using a Cambridge filter and DPBS. Although this study leveraged DMSO to facilitate the effective combination of HTP's particulate and gaseous phases, the potential solvent impact of DMSO warrants further investigation.

The HTPAC, developed herein utilizing 80 HTP sticks, can achieve an aerosol mass concentration of approximately 10 mg $stick^{-1}$ (16 mg mL^{-1} in DPBS; HTPAC volume = 50 mL) within a 5-hour timeframe. This concentration permits the dosing of over 500 Sprague-Dawley rats concurrently for toxicity tests via intratracheal instillation (The volume for a single injection is set at 100 μL). By diluting the HTPAC concentration, a greater number of rats can be administered doses. Thus, a single batch of HTPAC holds potential for both acute and sub-acute toxicity assessments. Moving forward, our research group aims to enhance the qualitative assessment and validation of the HTPAC, source HTPACs from a broader array of HTP types, and distribute them to toxicological assessment bodies, aiding in the formulation of safety guidelines for HTP usage.

CRediT authorship contribution statement

Yong-Hyun Kim: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft preparation, Writing – review & editing, Supervision. **Sung-Hwan Kim:** Formal analysis, Investigation, Data curation, Writing – original draft preparation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

Acknowledgements

This research was supported by a grant [grant number 23212MFDS252] from the Ministry of Food and Drug Safety in 2023 and a grant [grant number 2023-DD-RD-0140-01] from the Korea Innovation Foundation/Innopolis Foundation (the Ministry of Science and ICT) in 2023.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2023.115621.

References

Akiyama, Y., Sherwood, N., 2021. Systematic review of biomarker findings from clinical studies of electronic cigarettes and heated tobacco products. Toxicol. Rep. 8, 282–294.

AlOkda, A., Van Raamsdonk, J.M, 2022. Effect of DMSO on lifespan and physiology in C. elegans: Implications for use of DMSO as a solvent for compound delivery. MicroPubl. Biol. 2022. ⟨https://www.micropublication.org/static/pdf/micropub-biology-000634.pdf⟩.

An, Y.-J., Choi, S.-J., Kim, Y.-H., Lee, K., 2019. Quantitative Characteristics of Toxic Compounds According to the Solvent Type. J. Anal. Methods Chem., 3201370

Aranyosi, J.K., Galgoczi, E., Erdei, A., Katko, M., Fodor, M., Ujhelyi, Z., Bacskay, I., Nagy, E.V., Ujhelyi, B., 2022. Different effects of cigarette smoke, heated tobacco product and e-cigarette vapour on orbital fibroblasts in graves' orbitopathy; A study by real time cell electronic sensing. Molecules 27 (9), 3001.

Bansal, V., Kim, K.-H., 2016. Review on quantitation methods for hazardous pollutants released by e-cigarette (EC) smoking. TrAC, Trends Anal. Chem. 78, 120–133.

Başaran, R., Güven, N.M., Eke, B.C., 2019. An overview of iQOS® as a new heat-not-burn tobacco product and its potential effects on human health and the environment. Turk. J. Pharm. Sci. 16 (3), 371–374.

Becker, L.C., Bergfeld, W.F., Belsito, D.V., Hill, R.A., Klaassen, C.D., Liebler, D.C., Marks Jr, J.G., Shank, R.C., Slaga, T.J., Snyder, P.W., 2019. Safety assessment of glycerin as used in cosmetics. Int. J. Toxicol. 38 (3_suppl), 6S–22S.

Behr, J., Nowak, D., 2022. Tobacco smoke and respiratory disease. In the Impact of Air Pollution on Respiratory Tract. Monograph 21. ERS J. LTD Vol. 7, 161–180 (Available at). ⟨https://www.researchgate.net/profile/Dennis-Nowak-2/publication/251883915_Tobacco_smoke_and_respiratory_disease/links/547085f10cf24af340c0bfeb/Tobacco-smoke-and-respiratory-disease.pdf⟩.

Belson, M., Morgan, B.W., 2004. Methanol toxicity in a newborn. J. Toxicol. Clin. Toxicol. 42 (5), 673–677.

Bhat, T.A., Kalathil, S.G., Leigh, N., Muthumalage, T., Rahman, I., Goniewicz, M.L., Thanavala, Y.M., 2021. Acute effects of heated tobacco product (IQOS) aerosol inhalation on lung tissue damage and inflammatory changes in the lungs. Nicotine Tob. Res. 23 (7), 1160–1167.

Bialous, S.A., Glantz, S.A., 2018. Heated tobacco products: another tobacco industry global strategy to slow progress in tobacco control. Tob. Control 27 (Suppl 1), s111–s117.

Brayton, C.F., 1986. Dimethyl sulfoxide (DMSO): a review. Cornell Vet. 76 (1), 61–90.

Camoni, N., Conti, G., Esteves-Oliveira, M., Carvalho, T.S., Roccuzzo, A., Cagetti, M.G., Campus, G., 2023. Electronic cigarettes, heated tobacco products, and oral health: A systematic review and meta-analysis. Appl. Sci. 13 (17), 9654.

Cancelada, L., Sleiman, M., Tang, X., Russell, M.L., Montesinos, V.N., Litter, M.I., Gundel, L.A., Destaillats, H., 2019. Heated tobacco products: volatile emissions and their predicted impact on indoor air quality. Environ. Sci. Technol. 53 (13), 7866–7876.

Carll, A.P., Arab, C., Salatini, R., Miles, M.D., Nystoriak, M.A., Fulghum, K.L., Riggs, D. W., Shirk, G.A., Theis, W.S., Talebi, N., Bhatnagar, A., Conklin, D.J., 2022. E-cigarettes and their lone constituents induce cardiac arrhythmia and conduction defects in mice. Nat. Commun. 13, 6088.

Chen-Sankey, J.C., Kechter, A., Barrington-Trimis, J., McConnell, R., Krueger, E.A., Cruz, T.B., Unger, J.B., Chaffee, B.W., Leventhal, A., 2023. Effect of a hypothetical modified risk tobacco product claim on heated tobacco product use intention and perceptions in young adults. Tob. Control 32 (1), 42–50.

Crosswhite, M.R., Bailey, P.C., Jeong, L.N., Lioubomirov, A., Yang, C., Ozvald, A., Jameson, J.B., Gillman, I.G., 2021. Non-targeted chemical characterization of Juul Virginia tobacco flavored aerosols using liquid and gas chromatography. Separations 8 (9), 130.

DeMarini, D.M., Gudi, R., Szkudlinska, A., Rao, M., Recio, L., Kehl, M., Kirby, P.E., Polzin, G., Richter, P.A., 2008. Genotoxicity of 10 cigarette smoke condensates in four test systems: comparisons between assays and condensates. Mutat. Res. - Genet. Toxicol. Environ. Mutagen. 650 (1), 15–29.

Dusautoir, R., Zarcone, G., Verriele, M., Garçon, G., Fronval, I., Beauval, N., Allorge, D., Riffault, V., Locoge, N., Lo-Guidice, J.-M., 2021. Comparison of the chemical composition of aerosols from heated tobacco products, electronic cigarettes and tobacco cigarettes and their toxic impacts on the human bronchial epithelial BEAS-2B cells. J. Hazard. Mater. 401, 123417.

East, K.A., Tompkins, C.N., McNeill, A., Hitchman, S.C., 2021. I perceive it to be less harmful, I have no idea if it is or not:'a qualitative exploration of the harm perceptions of IQOS among adult users. Harm Reduct. J. 18 (1), 12.

Farsalinos, K.E., Yannovits, N., Sarri, T., Voudris, V., Poulas, K., Leischow, S.J., 2018. Carbonyl emissions from a novel heated tobacco product (IQOS): comparison with an e-cigarette and a tobacco cigarette. Addiction 113 (11), 2099–2106.

Gasparyan, H., Mariner, D., Wright, C., Nicol, J., Murphy, J., Liu, C., Proctor, C., 2018. Accurate measurement of main aerosol constituents from heated tobacco products (HTPs): implications for a fundamentally different aerosol. Regul. Toxicol. Pharmacol. 99, 131–141.

Helen, G.S., Jacob Iii, P., Nardone, N., Benowitz, N.L., 2018. IQOS: examination of Philip Morris International's claim of reduced exposure. Tob. Control 27 (Suppl 1), s30–s36.

Hirano, T., Shobayashi, T., Takei, T., Wakao, F., 2020. Exposure assessment of environmental tobacco aerosol from heated tobacco products: nicotine and PM exposures under two limited conditions. Int. J. Environ. Res. Public Health 17 (22), 8536.

Hoyberghs, J., Bars, C., Ayuso, M., Van Ginneken, C., Foubert, K., Van Cruchten, S., 2021. DMSO concentrations up to 1% are safe to be used in the zebrafish embryo developmental toxicity assay. Front. Toxicol. 3, 804033.

Jafvert, C.T., Kulkarni, P.P., 2008. Buckminsterfullerene's (C60) octanol− water partition coefficient (K ow) and aqueous solubility. Environ. Sci. Technol. 42 (16), 5945–5950.

Jankowski, M., Brożek, G.M., Lawson, J., Skoczyński, S., Majek, P., Zejda, J.E., 2019. New ideas, old problems? Heated tobacco products–A systematic review. Int. J. Occup. Med. Environ. Health 32 (5), 595–634.

Kawai, T., Zhang, Z.-W., Takeuchi, A., Miyama, Y., Sakamoto, K., Higashikawa, K., Ikeda, M., 2003. Methyl isobutyl ketone and methyl ethyl ketone in urine as biological markers of occupational exposure to these solvents at low levels. Int. Arch. Occup. Environ. Health 76, 17–23.

Kim, J.S., Kim, O.-H., Ryu, I.S., Kim, Y.-H., Jeon, K.O., Lim, L.-N., Kim, T.W., Sohn, S., Kim, S., Seo, J.-W., 2022a. Challenge exposure to whole cigarette smoke condensate upregulates locomotor sensitization by stimulating α4β2 nicotinic acetylcholine receptors in the nucleus accumbens of rats. Pharmacol. Biochem. Behav. 220, 173469.

Kim, K.-H., Kim, Y.-H., Brown, R.J., 2013. Conditions for the optimal analysis of volatile organic compounds in air with sorbent tube sampling and liquid standard calibration: demonstration of solvent effect. Anal. Bioanal. Chem. 405, 8397–8408.

Kim, S., Sohn, S., Ryu, I.S., Yang, J.H., Kim, O.-H., Kim, J.S., Kim, Y.-H., Jang, E.Y., Choe, E.S., 2022b. Nicotine rather than non-nicotine substances in 3R4F WCSC increases behavioral sensitization and drug-taking behavior in rats. Nicotine Tob. Res. 24 (8), 1201–1207.

Kim, Y.-H., An, Y.-J., 2020. Development of a standardized new cigarette smoke generating (SNCSG) system for the assessment of chemicals in the smoke of new cigarette types (heat-not-burn (HNB) tobacco and electronic cigarettes (E-Cigs)). Environ. Res. 185, 109413.

Kim, Y.-H., Kim, K.-H., 2015a. A novel method to quantify the emission and conversion of VOCs in the smoking of electronic cigarettes. Sci. Rep. 5, 16383.

Kim, Y.-H., Kim, K.-H., 2015b. Critical role of a pre-purge setup in the thermal desorption analysis of volatile organic compounds by gas chromatography with mass spectrometry. J. Sep. Sci. 38 (14), 2455–2462.

Kim, Y.-H., Kim, M.-S., 2023. Development and assessment of a novel standardized method for preparation of whole cigarette smoke condensate (WCSC) for toxicity testing of cigarette smoke. Microchem. J. 191, 108914.

Kim, Y.-H., An, Y.-J., Shin, J.-W., 2020. Carbonyl compounds containing formaldehyde produced from the heated mouthpiece of tobacco sticks for heated tobacco products. Molecules 25 (23), 5612.

Kopa, P.N., Pawliczak, R., 2020. IQOS–a heat-not-burn (HnB) tobacco product–chemical composition and possible impact on oxidative stress and inflammatory response. A systematic review. Toxicol. Mech. Methods 30 (2), 81–87.

Kostygina, G., Tran, H., Kim, Y., Czaplicki, L., Kierstead, E., Kreslake, J., Emery, S., Schillo, B., 2022. Heating Up: How Early Twitter Marketing Gave Rise to Organic Word-of-Mouth About Heated Tobacco Products. Soc. Media Soc. 8 (4), 20563051221141851.

Lau, Y.K., Okawa, S., Meza, R., Katanoda, K., Tabuchi, T., 2022. Nicotine dependence of cigarette and heated tobacco users in Japan, 2019: a cross-sectional analysis of the JASTIS Study. Tob. Control 31, e50–e56.

Lee, J.A., Lee, C., Cho, H.-J., 2023. Use of heated tobacco products where their use is prohibited. Tob. Control 32 (2), 146–152.

Leigh, N.J., Palumbo, M.N., Marino, A.M., O'Connor, R.J., Goniewicz, M.L., 2018. Tobacco-specific nitrosamines (TSNA) in heated tobacco product IQOS. Tob. Control 27 (Suppl 1), s37–s38.

Li, X., Luo, Y., Jiang, X., Zhang, H., Zhu, F., Hu, S., Hou, H., Hu, Q., Pang, Y., 2019. Chemical analysis and simulated pyrolysis of tobacco heating system 2.2 compared to conventional cigarettes. Nicotine Tob. Res. 21 (1), 111–118.

Lim, D.-H., Kim, Y.-H., Son, Y.-S., Jo, S.-H., Kim, K.-H., 2022a. A simple sampling method for quantification of hazardous volatile organic compounds in mainstream cigarette smoke: Method development and prestudy validation. Microchem. J. 180, 107602.

Lim, D.-H., Son, Y.-S., Kim, Y.-H., Kukkar, D., Kim, K.-H., 2022b. Volatile organic compounds released in the mainstream smoke of flavor capsule cigarettes. Environ. Res. 209, 112866.

Lim, D.-H., Ahmadi, Y., Kim, Y.-H., Kim, K.-H., 2023. The extent of harmful volatile organic compounds released when smoking after breaking the flavor capsules of heat-not-burn (HNB) cigarette products. Environ. Res. 216, 114501.

Logsdon, J.E., Loke, R.A., 2000. Isopropyl alcohol. Kirk-Othmer Encycl. Chem. Technol. https://doi.org/10.1002/0471238961.0919151612150719.a01.

Martin, A.E., Murphy, F.H., 2000. Glycols, propylene glycols. Kirk-Othmer Encycl. Chem. Technol. https://doi.org/https://onlinelibrary.wiley.com/doi/epdf/10.1002/0471238961.1618151613011820.a01.

Matsouki, N., Konstantinidis, E., Tsipa, C., Zervas, E., 2021. Comparison of carbonyls emitted from conventional cigarettes, electronic cigarettes and heated tobacco products, IOP Conference Series: Earth and Environmental Science. IOP Publishing, p. 012007.

Meehan-Atrash, J., Duell, A.K., McWhirter, K.J., Luo, W., Peyton, D.H., Strongin, R.M., 2019. Free-base nicotine is nearly absent in aerosol from IQOS heat-not-burn devices, as determined by 1H NMR spectroscopy. Chem. Res. Toxicol. 32 (6), 974–976.

Miller, C.R., Xu, S.S., Smith, D.M., Sutanto, E., Goniewicz, M.L., Quah, A.C., Fong, G.T., 2022. Assessing use of inhalable nicotine products within complex markets: the dilemma of heated tobacco products. BMJ Publ. Group Ltd.

Miranda, K., Dionísio, A.G.G., Neto, O.D.P., Gomes, M.S., Pereira-Filho, E.R., 2012. Determination of Cd levels in smoke condensate of Brazilian and Paraguayan cigarettes by Thermospray Flame Furnace Atomic Absorption Spectrometry (TS-FF-AAS). Microchem. J. 100, 27–30.

Montuschi, P., 2005. Exhaled breath condensate analysis in patients with COPD. Clin. Chim. Acta 356 (1–2), 22–34.

Morjaria, J.B., Campagna, D., Caci, G., O`Leary, R., Polosa, R., 2023. Health impact of e-cigarettes and heated tobacco products in chronic obstructive pulmonary disease: current and emerging evidence. Expert Rev. Respir. Med. 16 (11–12), 1213–1226.

Nakama, C., Tabuchi, T., 2021. Use of heated tobacco products by people with chronic diseases: The 2019 JASTIS study. Plos One 16 (11), e0260154.

NIOSH, 2019. NIOSH Pocket Guide to Chemical hazards. ⟨https://www.cdc.gov/niosh/npg/npgd0326.html#print⟩.

Nishimoto-Kusunose, S., Sawa, M., Inaba, Y., Ushiyama, A., Ishii, K., Hattori, K., Ogasawara, Y., 2022. Exposure to aerosol extract from heated tobacco products causes a drastic decrease of glutathione and protein carbonylation in human lung epithelial cells. Biochem. Biophys. Res. Commun. 589, 92–99.

Ono, Y., Takeuchi, Y., Hisanaga, N., 1981. A comparative study on the toxicity of n-hexane and its isomers on the peripheral nerve. Int. Arch. Occup. Environ. Health 48 (3), 289–294.

Onoue, A., Inaba, Y., Machida, K., Samukawa, T., Inoue, H., Kurosawa, H., Ogata, H., Kunugita, N., Omori, H., 2022. Association between Fathers' Use of Heated Tobacco Products and Urinary Cotinine Concentrations in Their Spouses and Children. Int. J. Environ. Res. Public Health 19 (10), 6275.

Pankow, J.F., Luo, W., Tavakoli, A.D., Chen, C., Isabelle, L.M., 2004. Delivery levels and behavior of 1, 3-butadiene, acrylonitrile, benzene, and other toxic volatile organic compounds in mainstream tobacco smoke from two brands of commercial cigarettes. Chem. Res. Toxicol. 17 (6), 805–813.

Park, E.-J., Park, Y.-J., Lee, S.J., Lee, K., Yoon, C., 2019. Whole cigarette smoke condensates induce ferroptosis in human bronchial epithelial cells. Toxicol. Lett. 303, 55–66.

Quinn Jr, M.J., Ziolkowski Jr, D., 2015. Wildlife toxicity assessment for triacetin. Wildlife toxicity assessments for chemicals of military concern. Elsevier,, pp. 291–301.

Rabenstein, A., Rahofer, A., Vukas, J., Rieder, B., Störzenhofecker, K., Stoll, Y., Burgmann, N., Pieper, E., Laux, P., Luch, A., Rüther, T., Mallock-Ohnesorg, N., 2023. Usage pattern and nicotine delivery during ad libitum consumption of pod e-cigarettes and heated tobacco products. Toxics 11 (5), 434.

Research And Markets, 2022. Global Heated Tobacco Products (HTP) Market: Analysis By Product Type (Leaf and Stick), Distribution Channel (Online and Offline), By Region Size and Trends with Impact of COVID-19 and Forecast up to 2027. pp. 1–157.

Rodrigo, G., Jaccard, G., Tafin Djoko, D., Korneliou, A., Esposito, M., Belushkin, M., 2021. Cancer potencies and margin of exposure used for comparative risk assessment of heated tobacco products and electronic cigarettes aerosols with cigarette smoke. Arch. Toxicol. 95 (1), 283–298.

Rubin, H., 2001. Synergistic mechanisms in carcinogenesis by polycyclic aromatic hydrocarbons and by tobacco smoke: a bio-historical perspective with updates. Carcinogenesis 22, 1903–1930.

Seo, H.G., Xu, S.S., Li, G., Gravely, S., Quah, A.C., Lee, S., Lim, S., Cho, S.-i, Kim, Y., Lee, E.S., 2023. Reasons for Initiation and Regular Use of Heated Tobacco Products among Current and Former Smokers in South Korea: Findings from the 2020 ITC Korea Survey. Int. J. Environ. Res. Public Health 20 (6), 4963.

Shaik, K.M., Sarmah, B., Wadekar, G.S., Kumar, P., 2022. Regulatory updates and analytical methodologies for nitrosamine impurities detection in sartans, ranitidine, nizatidine, and metformin along with sample preparation Techniques. Crit. Rev. Anal. Chem. 52 (1), 53–71.

Sun, H., Wang, S., Qi, S., Wang, P., Li, R., Shi, B., Zhang, Q., Huang, Q., Xu, S., Zhao, Y., 2023. Surface Defects Management by In Situ Etching with Methanol for Efficient Inverted Inorganic Perovskite Solar Cells. Adv. Funct. Mater., 2213913

Szukalska, M., Merritt, T.A., Lorenc, W., Sroczyńska, K., Miechowicz, I., Komorowicz, I., Mazela, J., Barałkiewicz, D., Florek, E., 2021. Toxic metals in human milk in relation to tobacco smoke exposure. Environ. Res. 197, 111090.

Tattan-Birch, H., Hartmann-Boyce, J., Kock, L., Simonavicius, E., Brose, L., Jackson, S., Shahab, L., Brown, J., 2022. Heated tobacco products for smoking cessation and reducing smoking prevalence. Cochrane Database Syst. Rev. (1).

Teague, S.V., Pinkerton, K.E., Goldsmith, M., Gebremichael, A., Chang, S., Jenkins, R.A., Moneyhun, J.H., 1994. Sidestream cigarette smoke generation and exposure system for environmental tobacco smoke studies. Inhal. Toxicol. 6 (1), 79–93.

Thun, M.J., Burns, D.M., 2001. Health impact of “reduced yield” cigarettes: a critical assessment of the epidemiological evidence. Tob. Control 10 i4-i11.

Uchiyama, S., Noguchi, M., Takagi, N., Hayashida, H., Inaba, Y., Ogura, H., Kunugita, N., 2018. Simple determination of gaseous and particulate compounds generated from heated tobacco products. Chem. Res. Toxicol. 31 (7), 585–593.

Wang, W., Yang, K., Sierra, J.M., Zhang, X., Yuan, S., Hu, Z., 2017. Potential impact of methyl isobutyl ketone (MIBK) on phenols degradation in an UASB reactor and its degradation properties. J. Hazard. Mater. 333, 73–79.

Yang, Y., Miller, D.J., Hawthorne, S.B., 1997. Toluene solubility in water and organic partitioning from gasoline and diesel fuel into water at elevated temperatures and pressures. J. Chem. Eng. Data 42 (5), 908–913.

Zhan, Y., Etter, J.F., Leischow, S., Zeng, D., 2019. Electronic cigarette usage patterns: a case study combining survey and social media data. J. Am. Med. Inform. Assoc. 26 (1), 9–18.

Zhu, S.-H., Ong, J., Wong, S., Cole, A., Zhuang, Y.-L., Shi, Y., 2022. Early adoption of heated tobacco products resembles that of e-cigarettes. Tob. Control 31 (e1), e35–e40.