

Editorial

# Method Development and Applications for Reduced-Risk Products

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## 1. Introduction

Cigarette smoking remains the leading cause of preventable premature death and disease in the U.S. There is an overwhelming scientific consensus that cigarette smoking is addictive and causes lung cancer, heart disease, chronic obstructive pulmonary disease, and other serious diseases [1]. While there are thousands of constituents in cigarette smoke, ref. [2] certain representative classes of chemicals characterized as harmful and potentially harmful constituents (HPHCs) have been studied extensively and attributed to the harm caused by the inhaled smoke of combusted tobacco [3]. Many people in the public health sector have acknowledged that a continuum of risk exists among tobacco products, with conventional combustible cigarettes at the highest end of that spectrum, and non-combustible products on the lower end [4–6]. In recent years, there has been rapid growth in the availability of innovative, non-combustible products, including oral tobacco-derived nicotine (OTDN) products, heated tobacco products (HTPs), and electronic cigarettes (also referred to as e-vapor products; EVPs). Because they are non-combustible, such products contain far fewer combustion-related HPHCs [7–9]. As a result, substantial reduction in the biomarkers for exposure to HPHCs have been reported among adult smokers who completely switch to such products [10,11]. Such large reductions in exposure to HPHCs are accompanied with favorable changes in biomarkers indicative of smoking-related disease outcomes [12]. Consequently, there is a growing body of evidence suggesting that such products likely present a substantial reduction in disease risks [13], and many people in the public health sector recognize the potential of such non-combustible products for reducing harm [6,14,15]. Therefore, switching to non-combustible alternatives presents a significant opportunity to decrease the burden of disease associated with smoking combustible cigarettes, particularly among adult smokers who are unable or unwilling to quit.

There is a growing body of research dedicated to characterizing non-combustible products. Many researchers from industry, academia, and government are working to develop and validate analytical methods to extract, separate, identify, and quantitate a variety of analytes from innovative tobacco products using a wide range of analytical techniques. Understanding the basic properties of these products is important to better characterize innovative oral and inhalable tobacco products. The oral non-combustible categories include traditional smokeless tobacco and OTDN products. Traditional smokeless tobacco products contain tobacco leaves and exist in three different forms including chewing tobacco (loose leaf, plug, or twist); snuff (finely ground tobacco that can be dry, moist, or packaged in pouches (e.g., snus)); and dissolvable (finely ground tobacco pressed into shapes such as tablets, sticks, or strips) products [16]. OTDN products, on the other hand, are tobacco-leaf free and are available in various forms including nicotine pouches, lozenges, gums, and dissolvable products [17,18]. These products may contain a number of ingredients that include tobacco-derived nicotine, pH adjusters (e.g., sodium carbonates), filler materials (e.g., modified cellulose, microcrystalline cellulose), sweeteners, stabilizers, and flavorings.



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Inhalable non-combustible products including EVPs and HTPs are compositionally different than cigarettes. Unlike traditional cigarettes, EVPs do not contain tobacco plant material or paper. They are mainly composed of a mixture of propylene glycol and glycerol in various ratios and flavors, and may or may not contain nicotine. In contrast, HTPs contain tobacco leaves but the tobacco is heated instead of burned, thereby lowering the temperature from  $>900\text{ }^{\circ}\text{C}$  to  $\sim 500\text{ }^{\circ}\text{C}$ . Due to the absence of tobacco leaves and paper in EVPs and the process of heating the tobacco in HTPs, many of the HPHCs in mainstream smoke are either not present or are present at significantly lower levels than smoking cigarettes [19,20].

The accurate determination and quantitation of constituents and chemicals in these products is needed for guiding product design, determining relative product performance, ensuring consistency during the manufacturing process, informing toxicological risk assessment, and regulatory reporting. This also allows for the characterization of inherent risks of innovative products, which helps determine whether the use of such products is potentially less harmful than smoking cigarettes. In this Special Issue, we discuss the latest analytical methods for chemical characterization of a variety of oral and inhalable non-combustible products.

## 2. Summary of Published Articles

This Special Issue includes research papers which address the latest analytical methods used for the identification and characterization of a variety of constituents and analytes in innovative oral and inhalable non-combustible tobacco products, using state-of-the-art techniques and instrumentations. The various contributions presented in this Special Issue are summarized based on the type of products evaluated and related methods reported.

Recently, nicotine pouches have emerged as a new category of innovative OTDN products. In this Special Issue, we received four contributions from different groups on methods that have been developed and validated to determine the nicotine release profiles, nicotine degradants, and HPHCs from a variety of nicotine pouch products. In these contributions, the authors have systematically used the developed methods to compare OTDN to traditional smokeless tobacco products. In the first manuscript, Aldeek et al. evaluated the nicotine release from 35 nicotine pouch products that are currently marketed in seven flavors with five different nicotine levels [21]. This is an important method to characterize the nicotine release from these pouches. The authors implemented a well-established dissolution method using the U.S. Pharmacopeia flow-through cell dissolution apparatus 4 (USP-4) that the same group previously developed for the evaluation of the nicotine release from traditional smokeless tobacco products [22]. The dissolution method was used for product-to-product comparison. The percent nicotine release profiles obtained from the 35 nicotine pouches under the same experimental conditions were found to be equivalent across all nicotine levels and flavors analyzed, indicating a similar rate of nicotine release from these oral nicotine pouch products. The authors further compared the percent nicotine release profiles from these nicotine pouches to a variety of other commercially available nicotine pouches and traditional pouched smokeless tobacco products. The authors state that the differences in percent nicotine release rates within the OTDN category could be associated with the inherent product characteristics (e.g., pouch paper and ingredients).

In the second manuscript, Knopp et al. developed a biorelevant dissolution method to study the nicotine release from OTDN nicotine pouches and portioned smokeless tobacco products (e.g., pouched snus) [23]. The *in vitro* release of nicotine was investigated in biorelevant volumes of artificial saliva using a custom-made dissolution apparatus. The apparatus consisted of a sinker that was prepared by 3D printing using polylactic acid material. The nicotine released was quantitated by a validated high-pressure liquid chromatography ultra-violet spectroscopy (HPLC-UV) method. The percent nicotine release profiles obtained from the OTDN and snus pouches were found to be distinct, indicating the ability of this method to discriminate between these two product categories. Additionally,

the authors compared the *in vitro* dissolution to *in vivo* data from a previously conducted clinical study [24]. Data showed a strong *in vitro*/*in vivo* correlation, indicating that the method reported in this publication is not only sensitive enough to discriminate between nicotine pouch and snus products, but could also serve as a predictive tool for product development and/or a monograph for oral tobacco/nicotine product equivalence studies.

The stability of nicotine depends on the inherent components of the product (e.g., fillers, pH, stabilizers, other ingredients, and moisture content) as well as the external environment (e.g., exposure to light and high temperatures). Therefore, developing methods to assess the nicotine stability in these products by monitoring the nicotine degradation compounds and select impurities is very important. These methods are useful to monitor the stability of nicotine in these products and for quality control purposes (e.g., to evaluate the purity of nicotine added to the product). In the third manuscript, Avagyan et al. developed a selective, accurate, and repeatable liquid chromatography–tandem mass spectrometry (LC-MS/MS) method for the determination of seven nicotine-related degradants and impurities [25]. The seven nicotine degradants in this method were nicotine-N'-oxide, cotinine, nor nicotine, anatabine, anabasine,  $\beta$ -nicotyrin, and myosmine. Most of the analytes were detected in the nicotine pouch products; however, they were found to be at lower levels compared to traditional tobacco products.

In the fourth manuscript, Jablonski et al. used fully validated CORESTA recommended methods to determine 17 selected HPHCs (including tobacco-specific nitrosamines, carbonyls, benzo[a]pyrene, nitrite, and metals) from 21 nicotine pouch products [26]. The selected pouches were obtained from seven different commercially available brands at the maximum nicotine level and a variety of flavors. The authors assessed two types of pouch products described as “white powder-based pouches” and “plant-based” pouches. The white powder-based pouches were similar to those described above, whereas the plant-based pouches were made from non-tobacco plant materials with pharmaceutical grade nicotine added during the production process. HPHCs in the 21 nicotine pouches were compared to those found in four traditional smokeless tobacco products (two CORESTA reference products and two commercially available products). The authors reported that the HPHCs levels, most notably metals, in the plant-based pouches were higher than those observed in powder-based products. In some plant-based pouches, these levels were even higher than those seen in traditional pouch smokeless tobacco products. However, the overall HPHCs levels observed in these plant-based nicotine pouches were at or below those levels observed in traditional pouch smokeless tobacco products.

The presence of unique constituents in the aerosol of EVPs is an important consideration in overall risk assessment of such products and is of interest to regulators and public health researchers. EVPs include both the e-liquid (containing nicotine and other ingredients) and aerosolizing apparatus, whether sold as a unit or separately. Due to the unique parts and components of EVPs, the constituents are distinct and specific to the product type (e.g., pod-based, open system, etc.). Therefore, in addition to the HPHCs, unknown compounds in the aerosol need to be characterized. The majority of analytical work on EVPs has focused on targeting known chemicals of interest based on changes to the device, formulation, power, temperature, or sampling approaches [27]. In this Special Issue, we received three contributions highlighting the development of targeted and non-targeted analytical methods for the determination of HPHCs and unknowns in EVPs. In the first report, Jin et al. evaluated the traditional 2,4-dinitrophenylhydrazine (2,4-DNPH) derivatization and quantitation of formaldehyde in e-liquid and aerosol of EVPs [28]. Formaldehyde is an HPHC listed by the FDA as a carcinogen and a respiratory toxicant [3]. Previous reports stated that formaldehyde is often underreported in EVPs due to a possible reaction with propylene glycol and glycerin in the aerosol which causes the formation of hemiacetals [29]. The research presented in this study provided a thorough experimental design to clearly demonstrate that hemiacetals formed in the aerosol readily hydrolyze to free formaldehyde and consequently form formaldehyde hydrazone in the typical 2,4-DNPH acidic trapping solution for quantitation. This study showed that the

commonly used 2,4-DNPH method is an appropriate method for the derivatization and accurate quantitation of formaldehyde in the aerosol generated by EVPs.

In the second manuscript, Chen et al. developed a comprehensive, targeted analysis using gas chromatography coupled to mass spectrometry (GC-MS) for the determination of 53 aerosol constituents from EVPs of currently marketed products [30]. The aerosol generation was conducted using non-intense and intense puffing regimens. Only 10 out of the targeted 53 analytes were quantifiable. The authors have compared their data to constituents collected from aerosols generated by both traditional cigarettes and a commercially available HTP that has been authorized for marketing in the U.S. The aerosol generated by the evaluated EVPs had detectable levels of ten targeted analytes including known degradants of propylene glycol and glycerin (e.g., acetaldehyde and formaldehyde) and nicotine-related compounds. The majority of tobacco-related HPHCs were not detectable in the aerosols. The levels of select HPHCs (other than nicotine) measured in the EVPs were found to be 96–99% lower than the same HPHCs reported in the cigarette smoke. However, the reduction levels of these select HPHCs in the EVPs ranged from 61% to 99% when compared to the levels found in HTP aerosol. The authors attributed the low levels of HPHCs in the EVPs' aerosols to the controlled temperature used in the device which is designed to reduce byproducts of combustion.

To address the potential gaps in understanding left by targeted analysis of EVPs, Crosswhite et al. developed and optimized liquid chromatography high resolution mass spectrometry (LC-HRMS) and GC-MS semi-quantitative methods to study unknown chemicals in generated aerosols [31]. These two methods were developed to account for the different physicochemical properties of possible chemical compounds including polarity, volatility, hydrophilicity, etc. The authors used differential analyses based on nine aerosol collection replicates of each studied EVP and each collection condition (intense and non-intense puffing regimens) to characterize compounds that differed from collection blanks. They relied on statistical tools to extract relevant information from a highly complex dataset. The authors reported all compounds at or above concentrations of 0.5 µg/g which were considered related to the sample. A total of 91 compounds were identified using these two methods in both non-intense and intense puffing regimens. This number was strikingly low when compared to the number of compounds (>5000) found in cigarette smoke [32]. Of the detected compounds, 47% were confirmed using reference standards. The authors showed that the studied aerosols from EVPs were approximately 50-fold less complex when compared to cigarette smoke.

We have also received two articles describing the development of LC-MS/MS methods for the identification of biomarkers of exposure specific to EVPs and other non-combustible products. Burkhardt et al. developed an LC-MS/MS method for measuring human exposure to 1,2-propylene glycol and glycerol, the main e-liquid constituents in EVPs [33]. These constituents were analyzed in plasma and urine samples from a clinical study comparing five nicotine product user groups (users of combustible cigarettes, EVPs, HTPs, oral tobacco products, and nicotine replacement therapy (NRT) products) and a control group of non-users. The results demonstrated elevated propylene glycol levels in urine and plasma in EVPs users compared to users of other products. The data showed a correlation between the propylene glycol and nicotine equivalents in the plasma and urine of EVP users. The nicotine equivalents were calculated by measuring the levels of nicotine and ten nicotine metabolites using a method developed by Piller et al. [34]. The authors also reported a dose–response relationship between urinary and plasma propylene glycol and intensity of vaping. The authors proposed that propylene glycol can be used as a potential biomarker to monitor compliance to EVP use when assessing switching behavior among smokers.

The same group, in a second article by Rogner et al., developed and validated another highly sensitive LC-MS/MS method for the determination of 3-hydroxybenzo[a]pyrene (3-OH-BaP), a metabolite of benzo[a]pyrene (BaP), in urine samples from smokers and non-combustible products users [35]. BaP is listed by FDA as an HPHC and classified

by IARC as a human carcinogen which is formed during the incomplete combustion of tobacco [3]. The method was validated with a very low limit of quantitation (50 pg/L) to account for trace levels of 3-OH-BaP in urine samples. The detected levels of 3-OH-BaP in urine samples were found to be significantly higher in cigarette smokers compared to non-combustible product users. The data presented by the authors showed the suitability of 3-OH-BaP as a biomarker for BaP and could be applied in clinical studies evaluating innovative non-combustible tobacco products.

### 3. Conclusions

The nine articles published in this Special Issue covered the latest analytical methods developed and applied for the chemical characterization or exposure assessment to tobacco product constituents of innovative non-combustible products (i.e., EVPs, HTPs, and OTDN products). The developed methods included (1) characterizing the nicotine dissolution release profiles and determining nicotine degradants and HPHCs in OTDN pouches; (2) identifying HPHCs, targeted, and unknown compounds in EVPs; and (3) determining potential biomarkers at trace levels in urine and blood samples in a variety of EVPs, HTPs, and OTDN products. The contributors to this Special Issue systematically compared the amount and release characteristics of select HPHCs, degradants, and unknown compounds found in innovative non-combustible products to combustible cigarettes or traditional smokeless tobacco products. This Special Issue is representative of the importance of analytical sciences research in characterizing innovative non-combustible products for guiding product design, determining relative product performance, ensuring consistency during the manufacturing process, informing toxicological risk assessment, and enabling regulatory reporting. The current advances in the development and applications of the analytical methods reported in this Special Issue can be used to inform the harm reduction potential of innovative non-combustible products for adult smokers.

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