Analyst

Accepted Manuscript

This article can be cited before page numbers have been issued, to do this please use: M. Finšgar and M. Rantaša, *Analyst*, 2026, DOI: 10.1039/D6AN00153J.

ROYAL SOCIETY OF CHEMISTRY

rsc.li/analyst

DOI: 10.1039/D6AN00153J

Flavourings, nicotine content, and elemental composition as indicators of regulatory compliance in nicotine pouches and heated tobacco products

Matjaž Rantaša[1], Matjaž Finšgar[1,*]

[1]University of Maribor, Faculty of Chemistry and Chemical Engineering, Smetanova ulica 17, 2000 Maribor, Slovenia

Abstract

Nicotine pouches (NPs) and heated tobacco products using heatsticks (HSs) are relatively new alternatives to traditional smoking. Their rising popularity, especially among younger populations, and recent regulatory efforts to reduce nicotine and tobacco use, emphasize the need for their analytical assessment. In this study, the chemical composition of 26 NPs and 16 HSs available on the Slovenian market has been investigated. Non-targeted gas chromatography-mass spectrometry analysis showed that some HSs contained compounds not permitted under regulations, including menthol, vanillin, and ethyl vanillin. NPs, which are not yet regulated regarding flavouring additives, contained potentially harmful compounds (cinnamaldehyde, acetaldehyde, menthofurane, pulegone, D-limonene, etc.), many of which were not declared on the product labels. Tobacco-based HSs contained significantly higher total element content (up to 2155.32 µg/g) compared to cellulose-based NPs (up to 61.21 µg/g), with Al, Ba, Fe, Mn, Sr, and Ti being the most abundant. Nicotine content ranged from 7.6 ± 0.2 mg/g of NP filling to 20.0 ± 0.1 mg/g NP filling in NPs and from 12.6 ± 0.1 mg/g of HS filling to 16.6 ± 0.1 mg/g of HS filling in HSs. The determined nicotine content in NPs deviated up to −52.9% from that declared by the manufacturers. These findings highlight the variability in the chemical composition of NPs and HSs, including their flavourings, nicotine content, and elemental composition, emphasizing the need for regulatory oversight and correct product labelling.

KEYWORDS: gas chromatography-mass spectrometry; heatsticks; inductively coupled plasma mass spectrometry; nicotine; nicotine pouches

* Corresponding author
Address: University of Maribor, Faculty of Chemistry and Chemical Engineering, Smetanova ulica 17, 2000 Maribor, Slovenia
e-mail: matjaz.finsgar@um.si; *phone*: +386 2 2294 447

View Article Online
DOI: 10.1039/D6AN00153J

1 Introduction

The use of tobacco and nicotine products remains a global public health concern. Despite numerous studies demonstrating their harmful health effects [1-3], and the impact of anti-smoking campaigns, which have contributed to a gradual decline in traditional tobacco use [4, 5], the problem persists. In recent years, novel products, including nicotine pouches (NPs) and heated tobacco products (HTPs), have entered the market. Their use is steadily increasing, particularly among younger age groups [6], who are often influenced by social media [7, 8], and among current smokers, who may perceive them as smoking cessation tools [9]. The global market for these products is rapidly expanding, with HTPs projected to reach USD 165.11 billion by 2030 [10] and NPs could reach up to USD 25.40 billion [11].

NPs are small pre-portioned pouches that vary in size and colour, typically containing a cellulose-based filler, nicotine or nicotine salts, preservatives, salts, pH adjusters, and flavourings [12]. They are placed between the gum and lip, where nicotine and other compounds are extracted by saliva and then absorbed into the body [13]. Their discreet use and absence of smoke contribute to their appeal. HTPs consist of a heating device and a tobacco-containing insert, commonly referred to as a heatstick (HS) [14]. During use, the HS is heated to approximately 350 °C, which is below the ignition point of tobacco, thereby delivering nicotine through aerosol without combustion [15]. Although HTPs and NPs are generally considered less harmful alternatives to traditional smoking [16], partly due to lower exposure to certain toxicants [17, 18], their health risks remain and are not yet fully understood. Reported adverse effects of HTP use include lung damage, chest pain, cardiovascular effects, inflammation, and shortness of breath [19-21]. Prenatal HTP use has been linked to increased allergy prevalence in newborns [22]. NPs may negatively affect oral health [23], increase heart rate [24], and, in some cases, cause acute nicotine poisoning [25]. Moreover, both HTPs and NPs are rapidly evolving in terms of composition and design in response to regulatory changes and consumer preferences, further complicating risk assessment.

In Slovenia, tobacco and nicotine products are regulated under the Restriction on the Use of Tobacco Products and Related Products Act (slo. *Zakon o omejevanju uporabe tobačnih in povezanih izdelkov*), most recently updated in 2024 [26]. This legislation aligns with the EU Tobacco Products Directive (2014/40/EU) and regulates cigarettes, electronic cigarettes, HTPs, and other nicotine-containing products. It specifies requirements for composition, packaging, labelling, sales restrictions, advertising bans, and flavour prohibitions. However, some products, notably NPs, remain only partially regulated, raising public health concerns [27]. While flavours and marketing of electronic cigarettes and HTPs are strictly limited, flavoured NPs are still legally available in Slovenia, increasing their appeal to young users and highlighting gaps in current legislation. According to data from the Slovenian National Institute of Public Health (2023), 30% of Slovenian high school students use electronic cigarettes, 12% use NPs, and 8% use HTPs [28]. These numbers are concerning, as early use of such products may increase the likelihood of later use of traditional cigarettes or cannabis, accelerate nicotine addiction in children, and potentially interfere with brain development. To address these issues, the Slovenian government has adopted a strategy called slo. *Strategija za zmanjševanje posledic rabe tobaka - Za Slovenijo brez tobaka 2022 – 2030*, which seeks to reduce tobacco consumption within the current decade [29, 30]. An even more ambitious goal is to eliminate tobacco and nicotine use entirely in Slovenia by 2040 [31]. Furthermore, in the field of nicotine and tobacco products, numerous initiatives and regulatory changes are occurring worldwide. These include efforts by the World Health Organization to promote measures to reduce the demand for tobacco [32], while, for example, in China, NPs are now regulated the same as cigarettes under China's state tobacco monopoly [33].

For these reasons, comprehensive chemical analysis is essential to evaluate the safety, quality, and potential health effects of NPs and HSs available in Slovenia. Such investigations are crucial, as the rapid introduction of these products outpaces the development of regulatory frameworks, leaving significant gaps in scientific knowledge and risk assessment. To our knowledge, no comparable study has been conducted in Slovenia or neighbouring countries, further emphasizing the importance of this

View Article Online
DOI: 10.1039/D6AN00153J

work. In this study, the chemical composition of NPs and HSs was analysed by gas chromatography-mass spectrometry (GC-MS), nicotine was quantified by GC-MS, elemental composition was determined by inductively coupled plasma-mass spectrometry (ICP-MS), and thermal behaviour was assessed using thermogravimetric analysis (TGA).

2 Experimental

2.1 Chemicals and reagents

A 50.0 mg/mL solution of nicotine in methanol (purchased from Lipomed GmbH, Weil am Rhein, Germany) and >98.0% quinoline (Tokyo Chemical Industry, Tokyo, Japan) were used for nicotine quantification. HPLC-grade tert-butyl methyl ether (MTBE) was supplied by Honeywell International (Muskegon, USA), while NaOH (Reag. Ph. Eur) was obtained from Riedel-de Haen (Seelze, Germany). NaCl (ACS grade) was acquired from Merck (Darmstadt, Germany), while Na_2SO_4 (≥99.99% trace metals basis) was purchased from Sigma Aldrich (St. Louis, Missouri, USA). A 100 mg/L multielement standard solution VIII and 1000 µg/mL single-element standards of As and Sn were supplied by Merck (Darmstadt, Germany), while 1000 µg/mL single-element standards of Ge, In, Mo, Re, Sc, and Ti were supplied by Inorganic Ventures (Christiansburg, Virginia, USA). For the acid digestion of samples, ultrapure 30–32% H_2O_2 (Carlo Erba reagents, Val-de-Reuil, France), superpure 29–31% HCl (Carlo Erba reagents, Val-de-Reuil, France), and suprapure 65% HNO_3 (Carl Roth, Karlsruhe, Germany) were used. All aqueous solutions were prepared using ultrapure water (resistivity 18.2 MΩ·cm) obtained using an ELGA PureLab water purification system (Veolia Water Technologies, United Kingdom).

2.2 Samples

NPs and HSs were purchased from local stores and kiosks in Maribor, Slovenia. In total, 26 NPs of various nicotine content and flavors and 16 HSs were obtained. NPs were assigned sample designations NP-X, HSs were assigned sample designations HS-X, while brands were assigned designations B-X, where X represents the sample or brand number (Table 1). Labelling information as specified by the manufacturer for NPs is provided in Table S1 (Supplementary Information), while different variations of some NPs are shown in Figure 1. NPs were stored in a freezer at −20 °C. Before analysis, they were transferred to a refrigerator at 4 °C for 24 h and finally equilibrated under ambient conditions [34]. HSs were stored in a dry and dark place at ambient conditions.

Table 1: Brands and their corresponding samples of NPs and HSs.

Brand		Sample designations
NPs	B-1	NP-1–NP-4
	B-2	NP-5–NP-14
	B-3	NP-15–NP-18
	B-4	NP-19–NP-22
	B-5	NP-23–NP-26
HSs	B-6	HS-1–HS-5
	B-7	HS-6–HS-9
	B-8	HS-10–HS-16

View Article Online
DOI: 10.1039/D6AN00153J

Figure 1: Photographs of the different variations of NPs (from top to bottom: NP-26, NP-20, and NP-18).

2.3 Moisture content and component mass

To determine the moisture content of NPs, they were weighed on an analytical balance and then dried to a constant mass in a TCF 50 Super laboratory dryer (Argo Lab, Carpi, Italy) at a temperature (*T*) of 99 °C [35]. The dried NPs were placed in a desiccator to prevent moisture adsorption from the atmosphere. Moisture content was calculated as the average mass difference of three replicates (Table S2). The dried NPs were then cut open, and the dried NP filling (NPF) was transferred into 50 mL centrifuge tubes.

HSs were first weighed and then cut open using a ceramic knife to isolate their individual components. The HS tobacco filling (HSF) was removed using plastic tweezers. Induction-heated HSs (Figure 2a) contained a metal susceptor, which was separated from the HSF using plastic tweezers, while blade-heated HSs (Figure 2b) did not contain a metal susceptor. All remaining HS components were weighed on an analytical balance (Table S3). The HSF was homogenized using a ceramic mortar and pestle, and the homogenized HSF was transferred into 50 mL centrifuge tubes for storage.

View Article Online
DOI: 10.1039/D6AN00153J

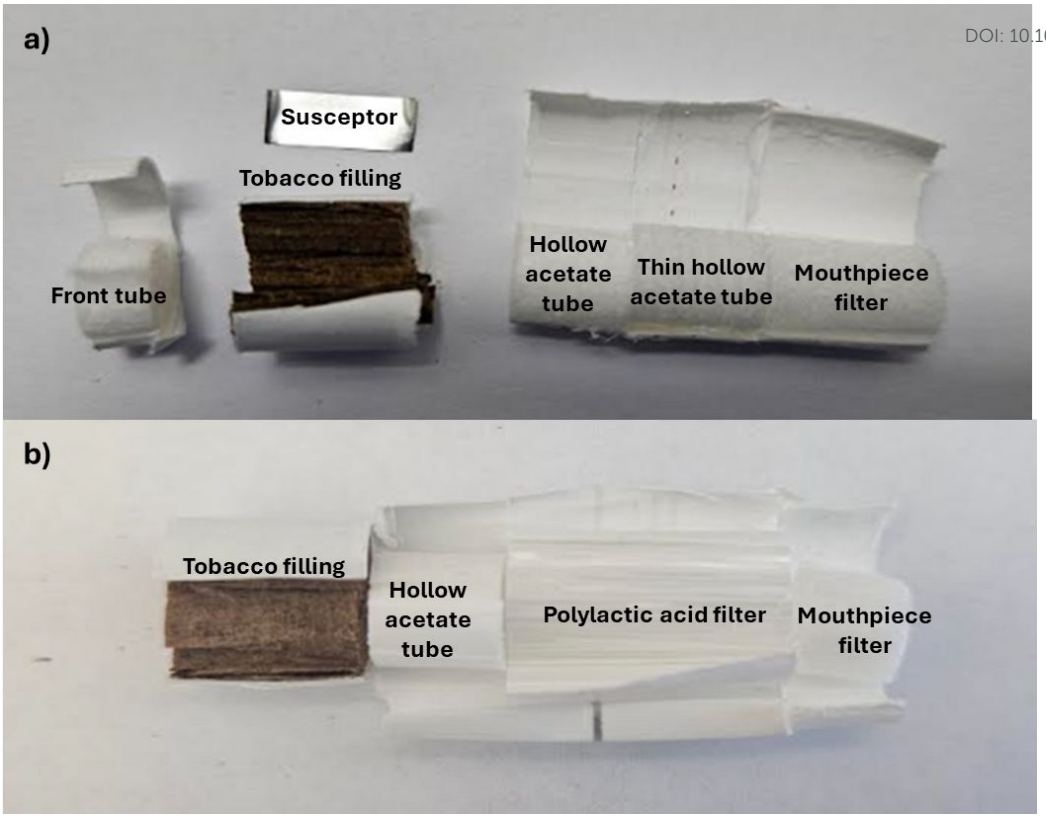

Figure 2: Photographs of HS components; a) induction-heated HS and b) blade-heated HS.

2.4 Gas chromatography-mass spectrometry

Gas chromatography (GC) analyses were performed using a GCMS-QP2020 NX instrument (Shimadzu, Kyoto, Japan) equipped with an AOC-6000 Plus autosampler (Shimadzu, Kyoto, Japan). Chromatographic compound separation was achieved on a 5% phenyl-arylene and 95% dimethylpolysiloxane (30 m, 0.25 mm, 0.25 µm) Zebron ZB-5MSplus capillary column (Phenomenex, Torrance, California, USA). Helium (Messer, Maribor, Slovenia), used as the carrier gas, was of 99.999% purity. A constant linear velocity of 36.1 cm/s and a column flow rate of 1.00 mL/min were used. Qualitative analyses were performed in a full scan mode over the mass-to-charge (*m/z*) range of 35–500.

2.4.1 Gas chromatography-mass spectrometry for HSs

Headspace solid-phase microextraction was employed as the sample introduction technique for the non-targeted analysis of HSs. Around 50 mg of homogenized HSF was weighed into 20 mL headspace glass vials. Four different sample preparation (SP) methods were tested, i.e. (i) sample preparation 1 (SP1): 50 mg of homogenized HSF, (ii) sample preparation 2 (SP2): 50 mg of homogenized HSF and 1 mL ultrapure water, (iii) sample preparation 3 (SP3): 50 mg of homogenized HSF, 1 mL ultrapure water, and 0.5 g NaCl (salting-out effect), and (iv) sample preparation 4 (SP4): 50 mg of homogenized HSF, 1 mL ultrapure water, and 0.5 g Na_2SO_4 (salting-out effect) [36, 37]. All mixtures were vortexed prior to the headspace solid-phase microextraction process.

Two Smart SPME fibers were used for the adsorption of volatile compounds a 95 µm Carbon Wide Range/PDMS and an 80 µm DVB/Carbon Wide Range/PDMS (Shimadzu, Kyoto, Japan). SPME fibers were conditioned at 270 °C for 10 min before and 5 min after each GC-MS analysis.

The samples were heated in an agitator at 95 °C for 10 min. The adsorption and desorption times were set to 5 min and 1 min, respectively, and the injector *T* was set to 260 °C. Measurements were performed using a 1:50 split ratio. The following oven *T* program was used: initial *T* of 40 °C, 20 °C/min increase to 130 °C, and 10 °C/min increase to 310 °C, resulting in a total runtime of 22.5 min. The MS interface *T* and ion source *T* were both set to 280 °C.

View Article Online
DOI: 10.1039/D6AN00153J

2.4.2 Gas chromatography-mass spectrometry for NPs

Whole NPs, without any pre-treatment, were placed in 20 mL headspace glass vials. The vials were heated in an agitator at 95 °C for 15 min. A headspace volume of 1000 µL was injected into the GC-MS instrument. The headspace syringe *T* and injector *T* were set to 100 °C and 260 °C, respectively. Measurements were performed using a 1:35 split ratio. The following oven *T* programme was applied: initial *T* of 40 °C, 6 °C/min increase to 100 °C, 5 min hold at 100 °C, 20 °C/min increase to 280 °C, resulting in a total runtime of 24.0 min. The MS interface *T* and ion source *T* were both set to 280 °C.

2.5 Nicotine extraction

For nicotine extraction, a modified version of the CORESTA recommended method No. 62 was used. This approach has previously been applied in several studies for nicotine extraction from both HSs and NPs, demonstrating satisfactory extraction efficiency for these samples [35, 38-41]. Briefly, 150 mg of homogenized HSF or 300 mg of NPF were weighed into 50 mL centrifuge tubes. Then, 7 mL of 2 N NaOH was added, and the mixture was allowed to stand for 15 min. Subsequently, 25 mL of MTBE that contained quinoline (internal standard) was added, and the samples were shaken on a rotary shaker at 200 revolutions per minute for two hours. The mixtures were then allowed to separate into organic and aqueous phases, and the upper MTBE phase was transferred into 1.5 mL glass vials. Each NP and HS was extracted in duplicate.

2.6 Nicotine quantification

Nicotine was quantified in MTBE extracts using the liquid injection sample introduction technique. The following oven *T* program was used: initial *T* of 40 °C, 20 °C/min ramp to 200 °C, and 40 °C/min ramp to 280 °C, resulting in a total runtime of 10 min. A solvent cut time of 3.00 min was used. Measurements were performed in selected ion monitoring (SIM) mode. For nicotine, *m/z* 84 was selected as a quantifier ion, while *m/z* 133 and 162 were selected as qualifier ions. For quinoline, a *m/z* of 129 was selected as a quantifier ion, and *m/z* of 76 and 102 were selected as qualifier ions [42]. A volume of 1 µL of each extract was injected using a 1:25 split ratio. Injector *T* was set to 260 °C, while both interface *T* and ion source *T* were set at 280 °C. Three replicate measurements were performed for each extract.

2.7 Microwave-assisted wet acid digestion

Samples were digested using an ETHOS EASY microwave system (Milestone, Sorisole, Italy). Approximately 200.0 mg of dried NPF was digested with 5.0 mL of 65 wt.% HNO_3. The following *T* was used: 20 min ramp to 230 °C, hold at 230 °C for 15 min. Approximately 200.0 mg of homogenized HSF was digested with 7.0 mL of 65 wt.% HNO_3, 2.0 mL of 35 wt.% HCl, and 1.0 mL of 30 wt.% H_2O_2. The following *T* was used: 20 min ramp to 210 °C, hold at 210 °C for 10 min, 5 min ramp to 230 °C, hold at 230 °C for 15 min. Digested samples were diluted to 50 mL with ultrapure water, filtered through polyamide syringe filters (0.45 µm pore size, 25 mm diameter) into 50 mL plastic centrifuge tubes, and then stored at 4 °C until analysis. A sample blank was prepared following the same procedure, without adding the respective sample material. For the spike recovery test, 125 µL of a 100 mg/L multielement standard and 125 µL of a prepared 100 mg/L solution of As, Mo, Ti, and Sn were added to the samples. All samples were prepared in triplicate.

2.8 Inductively coupled plasma mass spectrometry

The elemental composition of the digested samples was determined using an ICPMS-2030 (Shimadzu, Kyoto, Japan), equipped with an AS10 autosampler (Shimadzu, Kyoto, Japan). ICP-MS operating conditions are summarized in Table S4. The plasma argon gas (Messer, Maribor, Slovenia) was of 99.996% purity, while the helium gas, used in the collision cell to minimize polyatomic interferences, was of 99.999% purity.

A total of 18 elements were measured (Al, As, Ba, Cd, Co, Cr, Cu, Fe, Ga, Mn, Mo, Ni, Pb, Sb, Sr, Ti, Tl, and Zn). Ge, Sc, In, and Re were used as internal standards. To minimize cross-contamination, a 2 wt.% HNO_3 solution was injected between the next sample injection. Digested samples were additionally diluted with ultrapure water before analysis. To ensure matrix compatibility, the solutions of standards were prepared with acid concentrations matching those of the diluted digested samples.

2.9 Thermogravimetric analysis

Up to 50 mg of NPF or homogenized HSF were thermally decomposed using a TGA/DSC 3+ (Mettler Toledo, Columbus, Ohio, USA) TGA analyzer. Measurements were performed from 25 °C to 900 °C at a heating rate of 10 °C/min. A nitrogen flow (99.999% purity) of 40 mL/min was used to maintain an inert atmosphere. The released gaseous compounds were simultaneously measured using NicoletTM iS50 FT-IR (Thermo Fisher Scientific, Waltham, Massachusetts, USA) to obtain three-dimensional (3D) FTIR spectra. Both the transfer line *T* and the FTIR gas cell *T* were maintained at 250 °C to prevent the condensation of released volatiles [43]. Infrared spectra (IR) were measured in the 4000–500 cm^{-1} range.

3 Results and discussion

3.1 Mass and moisture content of HSs and NPs

To assess product characteristics relevant to product compliance, the mass and moisture content of NPs and HSs were evaluated. These parameters affect the concentration of nicotine and other constituents per consumption unit and therefore provide context for the analytical findings reported below. The results are reported as an average of replicates ($\bar{x}$) ± standard deviation (*s*).

The mass of NPs deviated from the declared values from −9.5% (NP-18) to 2.6% (NP-13), as shown in Table S2. The measured NP masses ranged from 400.7 ± 14.7 mg (NP-6) to 1,021.9 ± 23.5 mg (NP-1). NPs classified as mini NPs (NP-5–NP-7, NP-19, NP-20, NP-22) had the lowest masses, all below 500.0 mg. Slim NPs (NP-8–14, NP-21, NP-23, NP-24) had masses between 630.9 ± 176.6 mg and 819.2 ± 22.6 mg, whereas only one sample was labelled as regular (NP-25) with a mass of 991.5 ± 23.9 mg. The remaining NPs did not provide information regarding size or mass. The average pouch masses without the NPF ranged from 18.1 ± 1.2 mg (NP-3) to 74.2 ± 1.4 mg (NP-13). The moisture content of whole NPs ranged from 34.9 ± 0.5% (NP-7) to 55.1 ± 1.9% (NP-1), with an average moisture content of 43.9 ± 6.0% across all NPs. Six NPs had moisture content above 50.0%, including four NPs from B-1 (NP-1–NP-4), as well as NP-11 and NP-16.

The HS mass ranged from 583.1 ± 2.2 mg (HS-14) to 720.6 ± 1.3 mg (HS-9) as shown in Table S3. All HSs from B-8 had lower masses compared to HSs from B-6 and B-7. The HSF masses ranged from 238.2 ± 10.7 mg (HS-1) to 266.3 ± 11.0 mg (HS-11), while the filter mass (consisting of all the filters and tubes inside the HS; Figure 2) ranged from 96.9 ± 0.9 mg (HS-5) to 210.4 ± 4.7 mg (HS-10). The paper mass (paper, which surrounds the HS) ranged from 110.6 ± 1.6 mg (HS-14) to 137.9 ± 7.3 mg (HS-1), polyactic acid filter mass ranged from 224.3 ± 3.0 mg (HS-9) to 230.3 ± 7.5 mg (HS-4) and were present only in blade-heated HSs, while only induction-heated HSs contained susceptors, with masses ranging from 19.8 ± 0.2 mg (HS-14) to 20.9 ± 0.2 mg (HS-13).

3.2 Non-targeted gas chromatography-mass spectrometry analysis

Because product formulations can vary substantially and are not always fully reflected by labeling, GC-MS measurements were performed to determine the compounds present in the investigated NPs and HSs. This approach enables the identification of key flavourings and other relevant compounds across products. Compounds were identified using the NIST 17 Mass Spectral Library, applying a similarity index threshold of 90. The identified compounds for HSs are listed in Figure S1, and their relative areas normalized to 100% for every HS are given in Figure S2. The identified compounds for NPs are listed in Figures S3–S6, and their relative areas normalized to 100% for every NP are given in Figures S7–S10.

View Article Online
DOI: 10.1039/D6AN00153J

3.2.1 Non-targeted gas chromatography-mass spectrometry analysis of HSs

To improve the non-targeted GC-MS analysis of HSs, the effects of SPs and SPME fibres on extraction performance were evaluated. Performance was compared based on chromatographic response and the number of detected compounds (chromatographic peaks) across the tested conditions. The best performance for non-targeted analysis of HSs was achieved using the 80 µm DVB/Carbon Wide Range/PDMS fiber in combination with SP1, which yielded the highest number of chromatographic peaks and the highest peak areas for most compounds [44].

In total, 25 compounds were identified in different HSs (Figure S1). Ten compounds were identified in all 16 HSs, including acetic acid, propylene glycol (PG), glycerol (GLY), menthol, glycerol 1,2-diacetate, triacetin, nicotine, hexadecane, neophytadiene, and 1,2,3-propanetriol 1-acetate. Other frequently identified compounds included 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (n = 15), propylene glycol 1-acetate (n = 14), benzyl alcohol (n = 13), and dodecane (n = 13). Especially nicotine, neophytadiene, triacetin, glycerol, and, in some HSs, menthol (HS-2, HS-7) and benzyl alcohol (HS-1, HS-10) had high chromatographic peak areas, suggesting higher concentration in HSs.

Humectants such as PG and GLY are added to cured tobacco during manufacturing to facilitate efficient heating and aerosol formation [45, 46]. Triacetin, with a retention time (t_R) of 7.46 min, a common HS additive, is intentionally added to improve aerosol delivery, while acetic acid (t_R = 1.67 min) may originate from the acetate tubes. Acetate esters such as glycerol 1,2-diacetate (t_R = 6.46 min), propylene glycol 1-acetate (t_R = 3.33), and 1,2,3-propanetriol-1-acetate (t_R = 5.12 min) may form during tobacco curing or due to thermal degradation and the esterification process during GC sample agitation. Several compounds exhibiting aromatic or sweet properties are generated during the curing of tobacco. The intense peak for neophytadiene (t_R = 12.94 min) signifies its higher content and is a known degradation product of chlorophyll and β-carotene [47]. The 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (t_R = 5.64 min) is a Maillard reaction product, which contributes to the characteristic sweet aroma of tobacco [48], while geranyl acetone (t_R = 8.72 min) is formed due to carotenoid degradation [49]. Nicotyrine (t_R = 9.16 min), identified in HS-3, is a nicotine dehydrogenation product [50]. Benzyl alcohol (t_R = 4.66 min), identified in 13 HSs, is commonly used as a solvent and possesses a fruity aroma, however, it may be harmful by inhalation at higher concentrations [51]. Additionally, both *cis*- (t_R = 6.23 min) and *trans*-4-tert-butylcyclohexanol (t_R = 6.37 min) stereoisomers were identified in three and nine HSs, respectively.

According to Slovenian legislation, the sale of HSs with a characteristic aroma (other than that of tobacco) resulting from the addition or combination of flavouring additives is prohibited. Vanillin (t_R = 8.23 min) and ethyl vanillin (t_R = 8.85 min) were identified in HS-12, while menthol (t_R = 5.98 min) was identified in all HSs. Overall, HSs predominantly contained compounds associated with tobacco and permitted additives, but were not compliant with regulations regarding flavouring additives.

3.2.2 Non-targeted gas chromatography-mass spectrometry analysis of NPs

Flavourings in NPs vary widely between products, making their chemical characterization essential for understanding what consumers are exposed to. A total of 163 compounds were identified in NPs (Figures S3–S6). When comparing brands, B-1, B-2, B-3, B-4, and B-5 had, on average, 42, 18, 21, 49, and 21 identified compounds, respectively, reflecting notable differences among manufacturers in achieving the desired flavour profiles of NPs. The highest number of compounds was identified in NP-20 with 62 compounds, followed by NP-19 with 57 compounds and NP-4 with 56 compounds. The lowest number of compounds was identified in NP-26, where 10 compounds were identified. The most frequently identified compounds were nicotine (n = 26, where n represents the number of samples), ethanol (n = 25), menthol (n = 25), D-carvone (n = 24), 1-butanol (n = 21), p-menthan-3-one (n = 21), D-limonene (n = 19), PG (n = 18), menthyl acetate (n = 16), and β-caryophyllene (n = 14). In addition to occurring more frequently, these compounds also exhibited the highest chromatographic peak areas (Figure S7–S10), indicating their potential for higher concentration in NPs.

View Article Online
DOI: 10.1039/D6AN00153J

Based on Regulation (EC) No. 1272/2008, regarding the classification, labelling, and packaging of substances and mixtures, compounds present at concentrations above certain thresholds (in terms of content) must be reported on the packaging, otherwise, they may not require labelling [52]. These thresholds depend on the hazard class of compounds and are typically set at ≥1% or ≥0.1% [52], with even lower limits applied to certain highly toxic compounds. The NPs analyzed in this study had 7 to 14 different compounds listed as main constituents. Additionally, the packaging of several NPs included warnings regarding the presence of certain compounds, such as mint oil (NP-6, NP-7, NP-8), hexen-2-al (NP-11), nicotine polacrilex (NP-12, NP-13, NP-14), 4-hydroxy-2,5-dimethyl-furan-2(3H)-on (NP-19, NP-22), (R)-p-mentha-1,8-diene also known as D-limonene (NP-20), and cinnnammaldehyde (NP-20). Among these, hexen-2-al, nicotine polacrilex, and 4-hydroxy-2,5-dimethyl-furan-2(3H)-on were not identified in any of the analyzed NPs by non-targeted GC-MS analysis.

D-limonene (t_R = 9.07 min), which imparts a citrusy flavour, was identified in 19 different NPs, including NP-20, where its presence was also reported on the packaging. This compound exhibits low oral toxicity and may cause skin irritation [53]. Cinnamaldehyde (t_R = 16.90 min), responsible for the characteristic cinnamon aroma, has been associated with adverse health effects [54] and was identified in one sample (NP-20), consistent with its labelling. Other aldehydes of concern included acetaldehyde (n = 6; t_R = 1.47 min), classified as possibly carcinogenic to humans (group 2B) by the International Agency for Research on Cancer (IARC) [55], and benzaldehyde (n = 8; t_R = 7.40 min), which imparts an almond flavour, but can cause respiratory tract irritation at elevated concentrations [56].

Mint oils (e.g., spearmint, peppermint) contain a variety of terpenes, terpenoids, terpene alcohols, and other volatile compounds, many of which were identified in NPs [57, 58]. The identified compounds associated with mint oils included menthol (n = 25), D-carvone (n = 24), D-limonene (n = 19), eucalyptol (n =12), β-pinene (n = 11), linalool (n = 10), 3-octanol (n = 10), γ-terpinene (n = 10), piperitone (n = 9), α-pinene (n = 8), pulegone (n = 8), β-myrcene (n = 8), β-bourbonene (n = 8), o-cymene (n = 8), sabinene hydrate (n = 7), α-terpineol (n = 6), terpinen-4-ol (n = 6), sabinene (n = 6), α-terpinene (n = 6), menthofurane (n =5), isopulegol (n = 4), γ-terpineol (n = 3), cis-β-ocimene (n = 2), β-copaene (n = 2), and β-terpineol (n = 2). These compounds primarily contribute to the herbal and woody aroma profiles, characteristic of mint-flavored NPs. Among the compounds associated with mint oil, several were identified as compounds of concern. IARC classifies β-myrcene (t_R = 8.04 min) as possibly carcinogenic to humans (category 2B) [59], while both D-carvone (t_R = 16.29 min) and linalool (t_R = 10.88 min), which imparts a lavender aroma, may cause allergic skin reactions [59]. Furthermore, several compounds are restricted from intentional addition to products according to Regulation (EC) No. 1334/2008 [60], namely menthofurane (n = 5; t_R = 13.12 min) and pulegone (n = 8; t_R = 16.13 min), which is possibly carcinogenic to humans.

The peculiar aromas of NPs are influenced mainly by a variety of compounds, particularly esters and alcohols. Esters, which typically give a sweet and fruity aroma included methyl acetate (n = 16), ethyl butanoate (n = 8), ethyl 2-methyl butanoate (n = 8), hexyl acetate (n = 8), cis-3-hexenyl acetate (n = 7), butyl acetate (n = 6), ethyl hexanoate (n = 6), isopentyl isopentanoate (n = 4), benzyl acetate (n = 3), bornyl acetate (n = 3), isobutyl acetate (n = 2), ethyl 3-methyl butanoate (n = 2), 3-methyl-1-butyl acetate (n = 2), 2-methyl-1-butyl acetate (n = 2), 3-octanyl acetate (n = 2), hexyl hexanoate (n = 2), methyl cinnamate (n = 2) and several others. Alcohols included a variety of aromatic and non-aromatic alcohols such as ethanol (n = 25) 1-butanol (n = 21), *cis*-3-hexen-1-ol (n = 11), 3-octanol (n = 10), benzyl alcohol (n = 6), 1-hexanol (n = 4), 3-methyl-1-butanol (n = 3), 2-methyl-1-propanol (n = 2), and 2-methyl-1-butanol (n = 2). Ethanol is commonly used as a solvent to extract nicotine from tobacco leaves, and its presence may therefore be attributed to this process [61]. An interesting compound to highlight is N-ethyl-p-menthane-3-carboxamide, commonly known as WS-3 (t_R = 20.48 min) [62, 63]. This synthetic cooling agent, generally recognized as safe for consumption, was identified in three NPs (NP-1, NP-2, and NP-25).

Overall, flavored NPs might elicit local toxicological responses, as suggested by some studies [64], with the potential risk of adverse health effects increasing with the number and concentration of flavoring compounds. Conversely, other studies report that artificial saliva extracts of NPs cannot be

View Article Online
DOI: 10.1039/D6AN00153J

classified as irritants and are less likely to cause harm than some other nicotine products [65]. Nevertheless, NP labels should include a more comprehensive list of compounds, given the high number of flavorings present in such products, many of which are not currently disclosed. Current regulations also primarily focus on limit values for individual compounds, without addressing cumulative exposure to flavorings present in NPs, especially during prolonged contact with the oral mucosa.

3.3 Nicotine content

Nicotine content is a primary parameter for evaluating these products because it directly determines consumer exposure and enables comparison with reported nicotine values. Therefore, nicotine was quantified in the investigated NPs and HSs using the GC-MS method described in Section 2.6. The method for nicotine quantification was validated in accordance with ICH guidelines [66]. The t_R for quinoline and nicotine were 6.38 min and 7.14 min, respectively. Due to the heteroscedasticity of the variances of replicate responses measured at different calibration concentrations, as indicated by both the Bartlett and Hartley tests, a weighted linear regression model was employed to generate the calibration curve [67]. A weighting factor of $1/y_i^2$ (where y_i represents the ratio of nicotine intensity to quinoline intensity) was applied, as it had the lowest sum of the absolute values of the relative errors of 21.9% (Table S5). The determined linear concentration range was from 10.0 mg/L to 310.0 mg/L with a correlation coefficient of 0.9979. The accuracy and precision of the method were evaluated using recovery (Re) and relative standard deviation (RSD) in %, respectively. For the spike recovery test, Re was 98.4% with RSD of 2.3%. Accuracy and precision were further assessed at three concentration levels within the determined linear concentration range, low (50.0 mg/L; Re = 94.9%, RSD = 0.1%), medium (150.0 mg/L; Re = 99.9%, RSD = 0.9%), and high (280.0 mg/L; Re = 94.4%, RSD = 2.2%). The limit of detection (LOD) and limit of quantification (LOQ) were determined based on the signal-to-noise ratio (*S*/*N*). The LOD for nicotine was 40 µg/L at *S*/*N* of 4.01, while the LOQ for nicotine was 130 µg/L at *S*/*N* of 12.90.

The nicotine content values are reported as $\bar{x} \pm s$. For HSs (Table S6), they are reported as both mg/g of HSF and mg/HS, and for NPs (Table S7), they are reported as both mg/g of NPF and mg/NP. These results are also presented in Figure 3a for HSs and in Figure 3b for NPs.

3.3.1 Nicotine content in HSs

The nicotine content in analyzed HSs was 14.8 ± 1.1 mg/g (average ± *s*) of HSF, corresponding to 3.7 ± 0.3 mg/HS, which is comparable to previously reported values for nicotine content in HSs (16.0 ± 1.0 mg/g of HSF [38] and 15.7 mg/g of HSF [68]). Among individual products, nicotine content ranged from 12.6 ± 0.1 mg/g of HSF (HS-5) to 16.6 ± 0.1 mg/g of HSF (HS-16), which corresponds to 3.1 ± 0.1 mg/HS (HS-5) and 4.2 ± 0.1 mg/HS (HS-16). When comparing brands, the average nicotine content increased in the order of B-6 (13.8 ± 1.0 mg/g HSF), B-7 (15.1 ± 1.2 mg/g HSF), and B-8 (15.3 ± 0.8 mg/g HSF).

The EU does not explicitly regulate nicotine content in HSs or in tobacco itself, to the best of our knowledge. The Tobacco Products Directive (2014/40/EU) regulates only the nicotine yield per cigarette, measured under standardized smoking conditions [69]. In 2025, the U.S. Food and Drug Administration (FDA) proposed limiting the nicotine content to 0.7 mg/g of tobacco in cigarettes, cigars, and cigarette tobacco, while HTPs would be exempt from this regulation [70]. Standardizing nicotine content in tobacco and tobacco products remains challenging because nicotine content is influenced by numerous factors, including tobacco type, cultivation conditions, and processing methods, which leads to significant variability among samples worldwide [71].

3.3.2 Nicotine content in NPs

The nicotine content in analyzed NPs was 13.8 ± 3.2 mg/g (average ± *s*) of NPF, however, similar values cannot be directly compared for individual NPs due to variability in pouch mass. Nicotine content among NPs ranged from 7.6 ± 0.2 mg/g of NPF (NP-5) to 20.0 ± 0.1 mg/g of NPF (NP-13),

consistent with recent reports [72]. Per unit of consumption, nicotine content ranged from 2.6 ± 0.1 mg/NP (NP-6) to 15.1 ± 0.1 mg/NP (NP-25). Among brands, the average nicotine content was 14.4 ± 2.4 mg/g of NPF (B-1), 13.8 ± 4.1 mg/g of NPF (B-2), 13.4 ± 3.7 mg/g of NPF (B-3), 11.9 ± 2.1 mg/g of NPF (B-4), and 15.8 ± 1.4 mg/g of NPF (B-5).

The measured nicotine content was generally lower than declared on the labels, with an average deviation of −28.8%. The lowest deviation from the declared value was −7.3% (NP-24), while the most significant deviation was −52.9% (NP-16).

As NPs are relatively new products, regulations governing their composition remain limited. Currently, no officially established nicotine content limit values for NPs exist in Slovenia, nor has a harmonized limit been established by the EU. Permissible values, therefore, vary between countries. For instance, the Czech Republic limits the nicotine content to 12 mg/NP [73], and the entire packet of 20 NPs must not contain more than 240 mg of nicotine, whereas Mallock et al. [74] proposed a limit of 16.7 mg/NP, as products with higher nicotine contents are considered acutely toxic when used orally. Furthermore, the FDA has established a list of authorized NPs that may be lawfully sold in the USA. Currently, only two brands are permitted with nicotine content of 3–9 mg/NP [75]. Consequently, products available in Europe can contain higher nicotine levels than those permitted in the USA. Among the analyzed NPs, none exceeded the limit value for acute toxicity of 16.7 mg/NP.

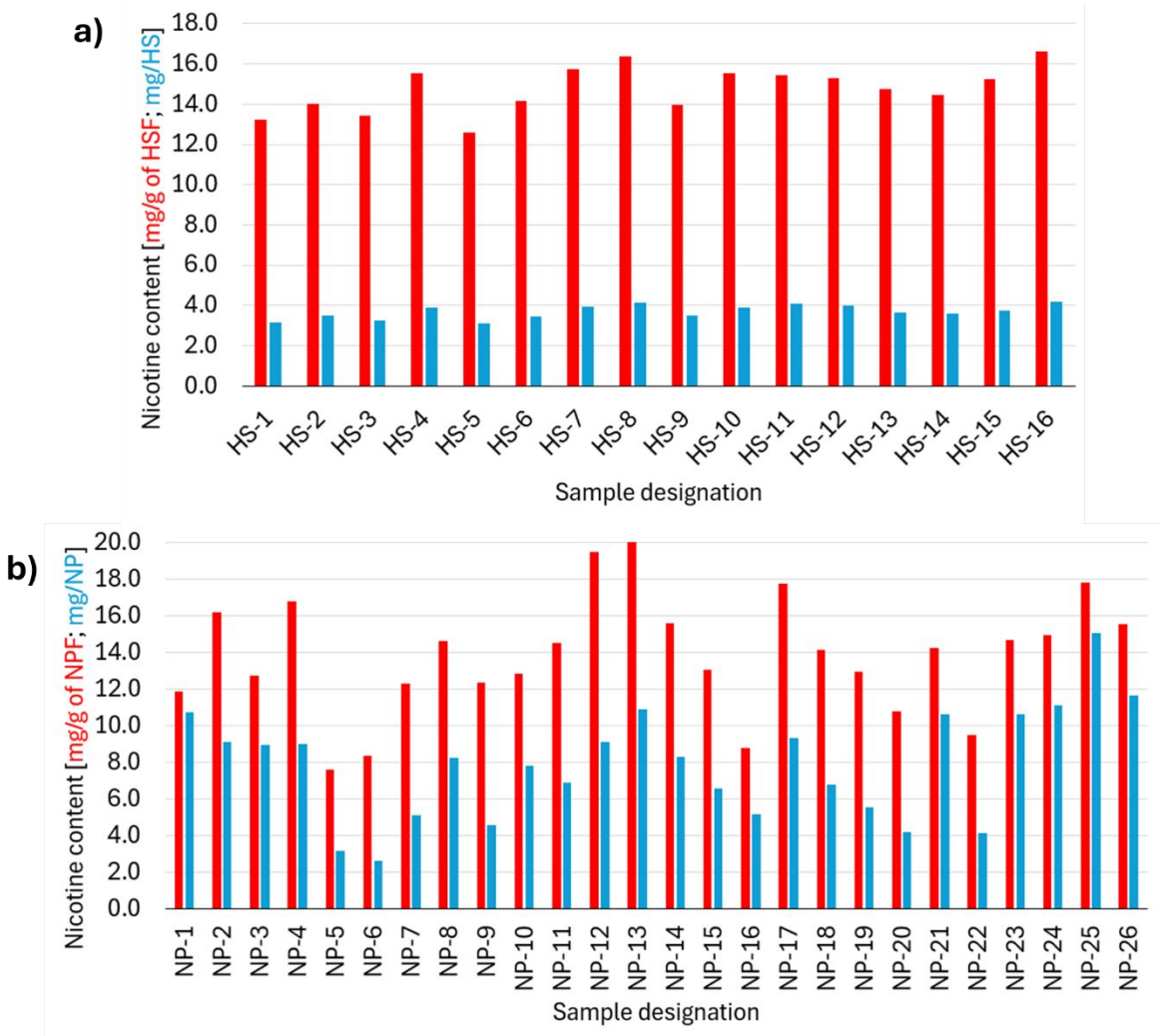

Figure 3: Nicotine content in different a) HSs and b) NPs.

3.4 Determination of elemental composition in NPs and HSs

Inorganic contaminants, including heavy metals and other elements, are relevant constituents of NPs and HSs due to their potential toxicity and variability among products. Therefore, the concentrations of selected elements in HSs and NPs were determined by ICP-MS. Heavy metals are naturally present in ingredients used for HSs and NPs. The tobacco plant accumulates heavy metals from the soil, therefore areas with contaminated soil may result in higher concentrations of heavy metals in tobacco. Additionally, the use of fertilizers and pesticides may introduce further contamination [76]. Microcrystalline cellulose, commonly used in NPs, is produced by the depolymerization of cellulose derived from biomass [77]. As biomass already naturally contains heavy metals, additional contamination can occur during processing. Furthermore, heavy metal contamination may originate from various stages of the manufacturing process or from the ingredients used [78].

ICP-MS validation parameters for 18 elements in HSs and NPs are reported in Table S8 and Table S9, respectively. For HSs, elemental composition is reported as µg/g of HSF and as µg/HS (Table S10). For NPs, the elemental composition is reported as µg/g of dried NPF and as µg/NP (Table S11). All results are reported as $\bar{x} \pm s$. Hereafter, elemental composition will be reported as µg/g (for HSs as µg/g of HSF and for NPs as µg/g of dried NPF), rather than per sample unit, due to variations in sample size, moisture content, and sample composition. For the spike recovery test, Re ranged from 86.2% to 102.9%, while the RSD of three replicates for the spike recovery test was below 6.9%, indicating both the accuracy and precision of the method, respectively.

Only a limited number of studies have reported elemental composition in NPs. Some studies focused on ICP-MS determination of elements such as As, Be, Cd, Co, Cr, Pb, Hg, Ni, and Se [79-81]. A similar lack of published data exists for HSs and HTPs [82-84].

The total element content (determined as the sum of masses of 18 elements per g of HSF or NPF and per unit of HS or NP) in all HSs and NPs is reported in Figure 4. On average, HSs exhibited a higher total element content compared to NPs. For HSs, the highest total element content was determined in HS-3 at 2155.32 µg/g, followed by HS-10 at 2018.26 µg/g. In contrast, only three NPs had a total element content above 50.00 µg/g, these being NP-24 with 51.48 µg/g, NP-25 with 58.19 µg/g, and NP-26 with 61.21 µg/g. Several NPs had the content for certain elements above LOQ but below the first calibration point of the linear concentration range (LOQ<C<CP1). When expressed per sample unit, the highest total element content among NPs was determined in NP-25 at 31.53 µg/NP and in NP-26 at 31.20 µg/NP, while among HSs it was determined in HS-3 at 524.39 µg/HS, followed by HS-11 at 519.35 µg/HS and HS-12 at 510.17 µg/HS.

As, Cd, Co, Pb, Sn, and Tl were below LOD in all NPs, while Mo was either below LOQ or below LOD. Al was quantified in five NPs (all four NPs from B-5 and NP-15) and was below LOQ in five other NPs. Al content ranged from 21.80 ± 0.90 µg/g (NP-23) to 32.08 ± 0.02 µg/g (NP-26), while all remaining NPs had Al content LOQ<C<CP1. Ba was quantified in 14 NPs, ranging from 0.46 ± 0.02 µg/g (NP-13) to 9.20 ± 0.28 µg/g (NP-21), while 12 had Ba content below LOQ. Cr was quantified in 22 NPs, ranging from 0.04 ± 0.01 µg/g (NP-26) to 0.70 ± 0.07 µg/g (NP-5), with three NPs below LOD and one NP LOQ<C<CP1. Cu was below LOD in 22 NPs, while it was quantified in all four samples from B-5, ranging from 1.32 ± 0.02 µg/g (NP-26) to 2.87 ± 0.08 µg/g (NP-24). Fe was quantified in 11 NPs, ranging from 10.74 ± 3.55 µg/g (NP-8) to 23.89 ± 1.42 µg/g (NP-25), while all other values were LOQ<C<CP1. Ga and Mn were quantified in all NPs, ranging from 0.11 ± 0.01 µg/g (NP-17) to 4.67 ± 0.17 µg/g (NP-21), and from 0.06 ± 0.03 µg/g (NP-7) to 1.20 ± 0.07 µg/g (NP-23), respectively. Ni was quantified in all but three NPs (NP-22 was below LOQ, while NP-24 and NP-26 were below LOD). Ni content ranged from 0.12 ± 0.11 µg/g (NP-25) to 2.90 ± 0.12 µg/g (NP-5), with only NP-5 exceeding 1.00 µg/g. Sr was quantified in all NPs, ranging from 0.11 ± 0.01 µg/g (NP-14) to 4.74 ± 0.01 µg/g (NP-20), closely followed by NP-22 with 4.70 ± 0.03 µg/g (both from B-4). Ti was quantified in 24 NPs from 0.21 ± 0.02 µg/g (NP-14) to 9.45 ± 0.01 µg/g (NP-11), which significantly exceeded the second-highest Ti content of 2.43 ± 0.17 µg/g (NP-2). Zn was below LOD in four NPs, below LOQ in one NP, and LOQ<C<CP1 in 21 NPs.

View Article Online
DOI: 10.1039/D6AN00153J

The element content of HSs indicated a similar composition across samples. Sn and Tl were below LOD in all HSs, while Pb was below LOD in two HSs (HS-2 and HS-16) and below LOQ in 14 other HSs. Mo was below LOD in six HSs, below LOQ in five HSs, and quantified in five HSs, ranging from 0.16 ± 0.03 µg/g (HS-13) to 0.23 ± 0.03 µg/g (HS-15). Cd was below LOQ in one HS (HS-16), while it ranged from 0.24 ± 0.01 µg/g (HS-7) to 0.57 ± 0.01 µg/g (HS-3). All other elements were quantified in every HS. Al content ranged from 696.14 ± 7.44 µg/g (HS-4) to 988.05 ± 42.22 µg/g (HS-5). As content ranged from 0.26 ± 0.01 µg/g (HS-7) to 0.39 ± 0.02 µg/g (HS-10 and HS-12), Ba content ranged from 52.15 ± 0.32 µg/g (HS-4) to 79.24 ± 4.45 µg/g (HS-3), Co content ranged from 0.70 ± 0.01 µg/g (HS-8) to 0.99 ± 0.06 µg/g (HS-3), Cr content ranged from 0.88 ± 0.03 µg/g (HS-7) to 3.25 ± 0.72 µg/g (HS-12), Cu content ranged from 7.69 ± 0.01 µg/g (HS-7) to 9.59 ± 0.40 µg/g (HS-3), Fe content ranged from 415.41 ± 1.29 µg/g (HS-4) to 671.41 ± 2.41 µg/g (HS-3), Ga content ranged from 27.25 ± 0.27 µg/g (HS-4) to 40.95 ± 2.44 µg/g (HS-3), Mn content ranged from 118.89 ± 0.67 µg/g (HS-4) to 176.62 ± 10.19 µg/g (HS-3). Ni content ranged from 1.13 ± 0.01 µg/g (HS-7) to 3.94 ± 0.59 µg/g (HS-12), closely followed by HS-10 and HS-11, with 3.92 ± 0.13 µg/g and 3.87 ± 0.11 µg/g, respectively. Sr content ranged from 76.89 ± 0.94 µg/g (NP-4) to 96.80 ± 1.48 µg/g (HS-8), Ti content ranged from 40.45 ± 0.07 µg/g (NP-4) to 68.96 ± 4.48 µg/g (NP-3), and Zn content ranged from 20.05 ± 0.42 µg/g (HS-12) to 27.81 ± 3.20 µg/g (HS-3).

The content of several elements in this study is consistent with values reported in previous studies conducted on tobacco from traditional cigarettes [85, 86]. HS-3 exhibited the highest content of Ba, Cd, Co, Cu, Fe, Ga, Mn, Ti, and Zn among all HSs.

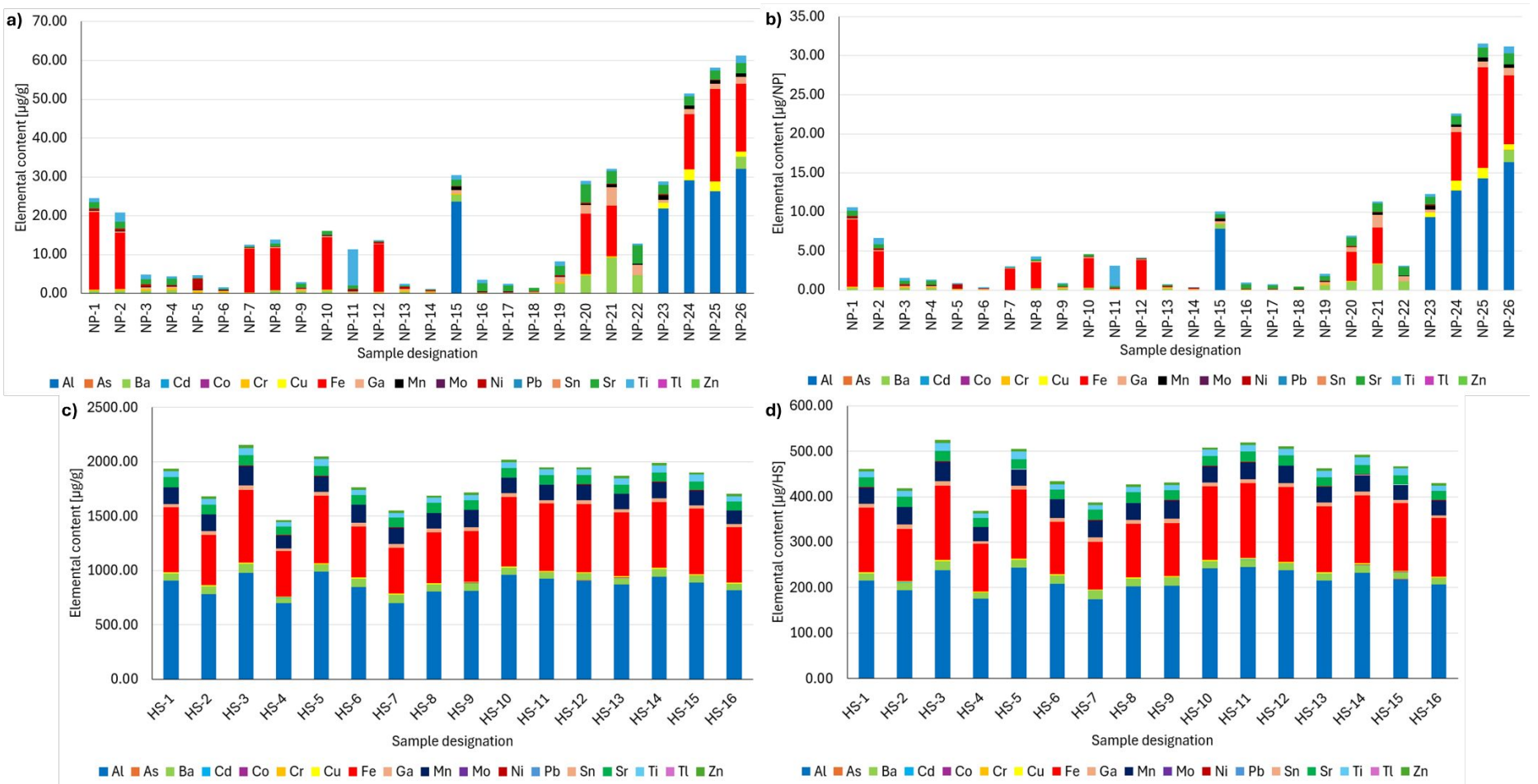

Figure 4: Total content of measured 18 elements in a) NPs reported as µg/g of dry NPF, b) NPs reported as µg/NP, c) HSs reported as µg/g of HSF, and d) HSs reported as µg/HS.

View Article Online
DOI: 10.1039/D6AN00153J

3.5 Thermogravimetric analysis of NPFs and HSFs

The TGA, derivative (DTG), and Gram-Schmidt (GS) curves, which represent the total IR intensity of gaseous compounds released during TGA, together with the 3-D FTIR spectra for each sample, are presented in Figures S11–S26 for HSFs and in Figures S27–S52 for NPFs.

The total mass loss of HSFs averaged 85.3%, ranging from 82.4% (HS-14) to 94.5% (HS-1). Based on the combined TGA and DTG curves for HSFs (Figure 5a and Figure 5b), multiple thermal decomposition steps were observed. The initial mass loss below 110 °C (6.9–10.8%) corresponds to the evaporation of moisture. The second stage, extending to approximately 240 °C, with a DTG peak near 210 °C (Figure 5b), was attributed to the evaporation of volatile compounds, such as nicotine, and to the thermal degradation of PG and GLY, which were identified by GC-MS analysis [43]. This stage represented 25.1–28.4% of the total mass. The highest mass loss (31.3%–39.3%) was attributed to the decomposition and pyrolysis of tobacco constituents, including hemicellulose, cellulose, lignin, and pectin [87]. Two distinct DTG peaks were observed in this stage, one below 270 °C, most likely associated with hemicellulose and pectin degradation, followed by a DTG peak at approximately 320 °C, corresponding to cellulose pyrolysis [88, 89]. The GS curve showed maximum absorbance at a similar *T*, confirming the formation of IR-active volatile compounds. A subsequent slower mass change contributed to approximately 3.8–5.7% of total mass, with the maximum decomposition rate near 470 °C, attributed to lignin degradation [43, 90]. The final stage (DTG peak around 660 °C) likely corresponds to char dehydrogenation [90]. Most HSFs exhibited mass losses of 5.0%–9.6% at this stage, except for HS-6, which had a mass loss of 12.5%.

The total mass loss of NPFs averaged 91.8%, ranging from 88.0% (NP-2) to 96.6% (NP-4). The TGA and DTG curves for NPFs (Figure 5c and Figure 5d) revealed a thermal behavior broadly similar to that of HSFs, although with greater variability among samples. The initial mass loss, attributed to the evaporation of moisture and low-boiling-point flavourings, was higher than in HSFs, ranging from 25.1% (NP-6) to 57.9% (NP-16). This was further supported by a mostly well-defined GS peak, especially in NPFs with high moisture content, whereas HSFs exhibited less pronounced GS peaks in this section. The second DTG peak was less intense compared to HSFs and corresponded to an additional mass loss, ranging from 2.5% (NP-10) to 14.1% (NP-15). This peak was clearly visible in some NPFs, while less distinct in others. The next thermal event was associated with the thermal degradation of microcrystalline cellulose, a primary constituent of NPs [80]. Its pyrolysis produced pronounced DTG and GS peaks, with a TGA mass loss between 270 °C and 380 °C (Figure 5d), accounting for 20.9% (NP-3) to 53.5% (NP-5) of the total mass. An additional DTG peak, accompanied by a high-intensity GS curve peak near 460 °C, was observed in seven of the 26 NPFs. These included three samples from B-2 (NP-12, NP-13, and NP-14), where nicotine polacrilex was reported on the product label, and all four samples from B-1 (NP-1–4), despite no such declaration. Nicotine polacrilex consists of nicotine bound to an ion-exchange resin (typically based on methacrylic acid and divinylbenzene), and it has been shown that divinylbenzene resins decompose at a similar *T* [91]. Therefore, this peak may indicate the presence of nicotine polacrilex in NPFs. The mass loss in this section was in the range from 4.6% (NP-13) to 7.4% (NP-2). The final thermal stage likely corresponded to char dehydrogenation and the decomposition of inorganic salts (K_2CO_3, Na_2CO_3, and $NaHCO_3$), which are declared as additives on the product labels [92]. These salts, used to maintain an alkaline pH, release CO_2 upon heating. The mass loss ranged from 2.1% (NP-23) to 13.7% (NP-4).

Qualitative information on the thermal degradation products of HSFs and NPFs and their functional groups was obtained from the 3-D FTIR spectra, as well as corresponding 2-dimensional (2-D) contour FTIR maps (Figure 6). For HSFs, most IR-active compounds appeared after approximately 20 min, while NPFs released fewer IR-active compounds during the first 15 min, with the majority evolving around 32 min of the TGA analysis. The main functional groups identified in the evolved gases were associated with O-H vibrations at 3500–3700 cm^{-1}, indicating the presence of water in samples. The low absorbance at the initial stage of TGA corresponded to the evaporation of physically adsorbed moisture, while the increase in absorbance after around 20 min most likely corresponded to the

View Article Online
DOI: 10.1039/D6AN00153J

release of water formed during the pyrolysis of organic tobacco constituents. In the range of 2830–3050 cm^{-1}, $-CH_2$ and $-CH_3$ vibrations were present, indicative of the potential formation of low-molecular-weight alkanes and alkenes. Peaks at 2250–2400 cm^{-1} can be attributed to the asymmetric C=O stretching, corresponding to the presence of CO_2. These peaks were observed in HSFs between 20 and 45 min, and in NPFs between 30 and 32 min. Furthermore, at 2100–2200 cm^{-1}, C-O vibrations, associated with CO formation, were observed. In HSFs, CO evolution appeared after 65 min, with relatively high absorbance intensities in some samples. NPFs exhibited similar but less intense absorbance bands around 30–32 min. The C=O stretching at 1798 cm^{-1} suggested the formation of compounds with carbonyl functional groups. Furthermore, peaks at 1400–1500 cm^{-1} represent C-H stretching, indicating the formation of methyl functional groups, while peaks at 1050–1200 cm^{-1} indicate C-O stretching, potentially indicating the release of alcohols and phenols [43, 90, 93].

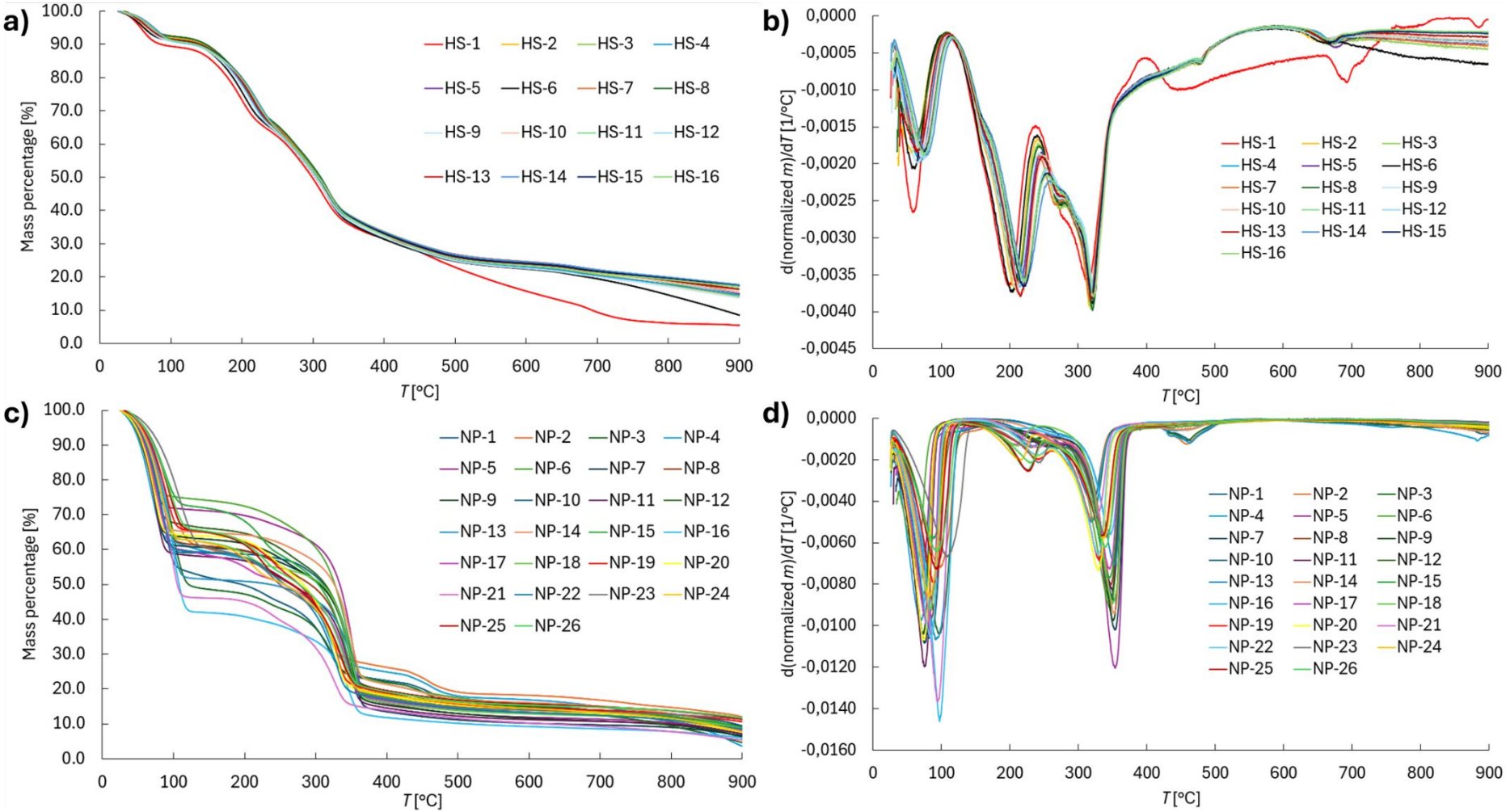

Figure 5: TGA analysis of 16 HSs and 26 NPs, a) TGA mass loss curves for HSFs, b) DTG curves for HSFs, c) TGA mass loss curves for NPFs, and d) DTG curves for NPFs.

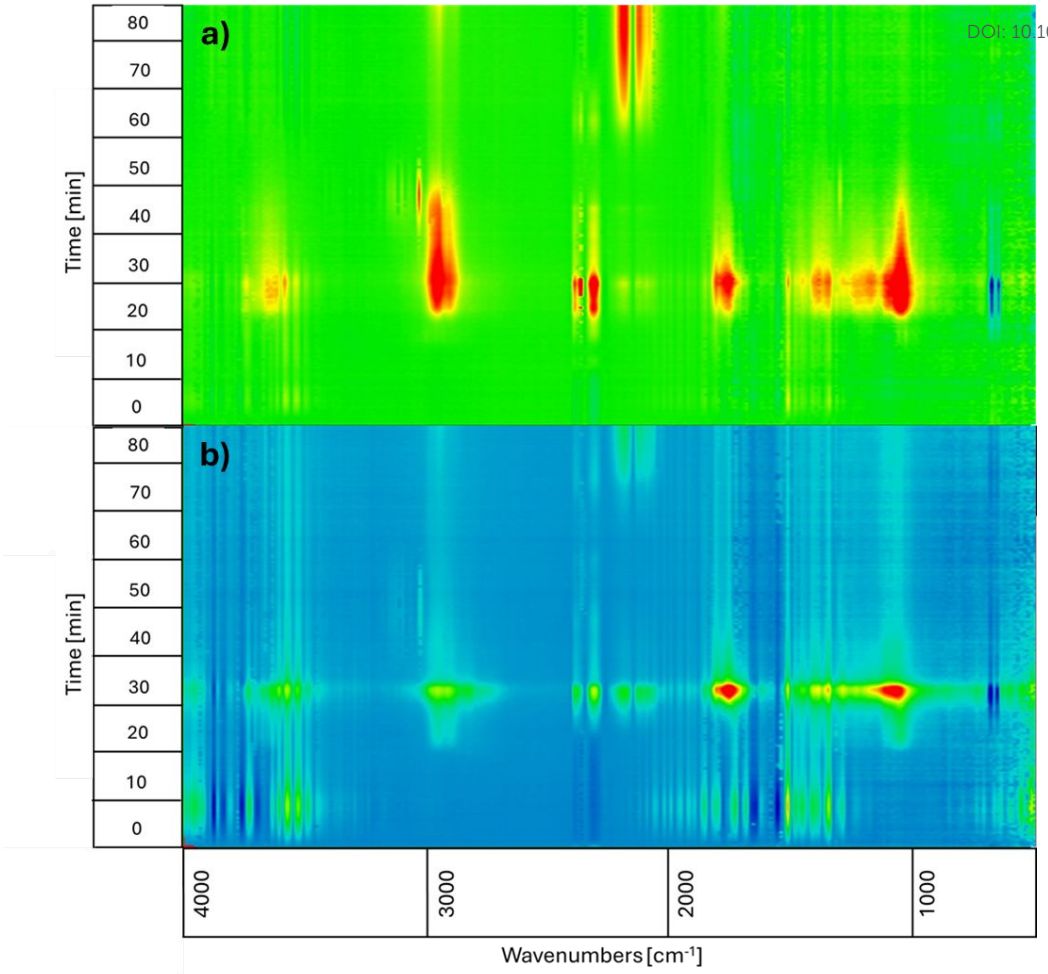

Figure 6: 2-D contour FTIR map for a) HSFs and b) NPFs.

4 Conclusions

In this study, nicotine pouches (NPs) and heatsticks (HSs) currently available on the Slovenian market were analysed regarding their chemical composition, moisture content, component weights, nicotine content, elemental composition, and their thermogravimetric profiles. Considerable variability was observed among NPs, whereas HSs exhibited a more consistent chemical composition. Several compounds not permitted under Slovenian legislation were identified in HSs, and a total of 163 compounds were identified in NPs, including some potentially harmful to human health. Most of these compounds were not declared on product labels, indicating a discrepancy between product formulation and consumer information. However, their concentration may fall below the regulatory thresholds required for mandatory labeling. These findings highlight the need to consider regulatory approaches similar to those applied to electronic cigarettes and heated tobacco products, where the use of flavourings is largely restricted. Nonetheless, it is essential to recognize that the health impact of flavourings on human health differs significantly when inhaled versus when used orally; therefore, such regulations may not be necessary.

HSs contained significantly higher concentrations of elements compared to cellulose-based NPs. However, both product types have the potential to introduce them into the human body through aerosol inhalation (HSs) or oral exposure (NPs). Nicotine content in HSs ranged between 12.6 ± 0.1 mg/g and 16.6 ± 0.1 mg/g in HS tobacco filling, while it ranged from 7.6 ± 0.2 mg/g to 20.0 ± 0.1 mg/g in NP filling. Deviations from manufacturer-reported values averaged −28.8%, emphasizing the importance of exact product labeling. Thermogravimetric analysis, combined with FTIR, revealed a multi-step decomposition process for both product types consisting of moisture loss, the release of

volatile compounds, and pyrolysis of the organic matrix, including tobacco (lignin, cellulose, and hemicellulose) in HSs and microcrystalline cellulose in NPs. Notably, certain NPs exhibited the potential presence of nicotine bound to ion-exchange resins, which may influence the kinetics of nicotine release during use.

Overall, these findings highlight the chemical complexity of both HSs and NPs as well as their potential health risks. Future research should focus on the toxicological assessment of identified flavourings, particularly those not declared on product labels, as well as long-term exposure to elements, especially heavy metals, arising from repeated use of HSs and NPs.

Author Contributions

Matjaž Rantaša: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – Original draft, Visualization **Matjaž Finšgar:** Conceptualization, Methodology, Resources, Writing – Review & Editing, Supervision, Project administration, Funding acquisition

All authors have read and agreed to the published version of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

The authors acknowledge the financial support of the Slovenian Research Agency (Grant Numbers: P2-0118, J2-50076, J1-4416, BI-RS/23-25-025, and GC-0007), and a young researcher fellowship for M.R.). The project is co-financed by the Republic of Slovenia, the Ministry of Higher Education, Science and Innovation, and the European Union under the European Regional Development Fund.

5 References

(1) Parmar, M. P.; Kaur, M.; Bhavanam, S.; Mulaka, G. S. R.; Ishfaq, L.; Vempati, R.; Kandepi, H. V.; Er, R.; Sahu, S.; Davalgi, S. A systematic review of the effects of smoking on the cardiovascular system and general health. *Cureus* **2023**, *15* (4).

(2) Hajat, C.; Stein, E.; Ramstrom, L.; Shantikumar, S.; Polosa, R. The health impact of smokeless tobacco products: a systematic review. *Harm Reduction Journal* **2021**, *18* (1), 123. DOI: 10.1186/s12954-021-00557-6.

(3) Rantaša, M.; Majer, D.; Finšgar, M. A review of analytical techniques for the determination of e-liquid and electronic cigarette aerosol composition. *Journal of Chromatography A* **2025**, *1748*, 465859. DOI: https://doi.org/10.1016/j.chroma.2025.465859.

(4) Organization, W. H. *WHO global report on trends in prevalence of tobacco use 2000–2030*; World Health Organization, 2024.

(5) Organization, W. H. Tobacco use declines despite tobacco industry efforts to jeopardize progress. *World Health Organization: Geneva, Switzerland* **2024**.

(6) Dai, H. D.; Leventhal, A. M. Prevalence of nicotine pouch use among US adults. *JAMA* **2024**, *332* (9), 755-757.

(7) Zenone, M.; Harries, B.; Hartwell, G. The promotion of oral nicotine pouches for non-smoking cessation purposes on TikTok. *Nicotine and Tobacco Research* **2025**, ntaf024.

(8) Gu, J.; Abroms, L. C.; Broniatowski, D. A.; Evans, W. D. An Investigation of Influential Users in the Promotion and Marketing of Heated Tobacco Products on Instagram: A Social Network Analysis. *Int. J. Env. Res. Public Health* **2022**, *19* (3), 1686.

(9) Alwafi, H.; Naser, A. Y.; Alharbi, R.; Tawakkul, S.; Albayhie, A.; Alsaiari, W.; Aloqilyi, J.; Althagafi, L.; Aldhahiri, A. M.; Alqarni, A. A.; et al. Prevalence, safety, and role of nicotine pouches in smoking cessation among smokers and the public in Saudi Arabia. *Sci. Rep.* **2025**, *15* (1), 29758. DOI: 10.1038/s41598-025-15070-0.

(10) *Heat-Not-Burn Market Size, Share & Trends Analysis Report By Component (Capsules, Devices, Loose-leaf, Sticks, Vaporizers, Others), By Distribution Channel (Online, Retail Store), By Region, And Segment Forecasts, 2024 - 2030*. 2023. https://www.grandviewresearch.com/industry-analysis/heat-not-burn-market (accessed 11.9.2025).
(11) *Nicotine Pouches Market Size, Share & Trends Analysis Report By Product Type (Tobacco-derived Nicotine, Synthetic Nicotine), By Distribution Channel (Offline, Online), By Flavor, By Strength, By Region, And Segment Forecasts, 2025 - 2030*. 2024. https://www.grandviewresearch.com/industry-analysis/nicotine-pouches-market-report (accessed 9.11.2025).
(12) Sparrock, L. S.; Phan, L.; Chen-Sankey, J.; Hacker, K.; Aniruddh, A.; Jewett, B.; Choi, K. Nicotine Pouch: Awareness, Beliefs, Use, and Susceptibility among Current Tobacco Users in the United States, 2021. *Int. J. Env. Res. Public Health* **2023**, *20* (3), 2050. DOI: https://doi.org/10.3390/ijerph20032050 Coronavirus Research Database; Publicly Available Content Database.
(13) Robichaud, M. O.; Seidenberg, A. B.; Byron, M. J. Tobacco companies introduce 'tobacco-free'nicotine pouches. *Tob. Control* **2020**, *29* (e1), e145-e146.
(14) Organization, W. H. Heated tobacco products: summary of research and evidence of health impacts. **2023**.
(15) Kopa, P. N.; Pawliczak, R. IQOS–a heat-not-burn (HnB) tobacco product–chemical composition and possible impact on oxidative stress and inflammatory response. A systematic review. *Toxicology mechanisms and methods* **2020**, *30* (2), 81-87.
(16) Pénzes, M.; Joó, T.; Urbán, R. Perceived harm of heated tobacco products, e-cigarettes, and nicotine replacement therapy compared with conventional cigarettes among ever and current heated tobacco users. *Addictive Behaviors Reports* **2022**, *15*, 100432. DOI: https://doi.org/10.1016/j.abrep.2022.100432.
(17) Grandolfo, E.; Ogden, H.; Fearon, I. M.; Malt, L.; Stevenson, M.; Weaver, S.; Nahde, T. Tobacco-Free Nicotine Pouches and Their Potential Contribution to Tobacco Harm Reduction: A Scoping Review. *Cureus* **2024**, *16* (2). DOI: https://doi.org/10.7759/cureus.54228 Publicly Available Content Database.
(18) Kusonić, D.; Bijelić, K.; Kladar, N.; Božin, B.; Torović, L.; Srđenović Čonić, B. Comparative health risk assessment of heated tobacco products versus conventional cigarettes. *Subst. Use Misuse* **2023**, *58* (3), 346-353.
(19) Bhat, T. A.; Kalathil, S. G.; Leigh, N.; Hutson, A.; Goniewicz, M. L.; Thanavala, Y. M. Do alternative tobacco products induce less adverse respiratory risk than cigarettes? *Respir. Res.* **2023**, *24* (1), 261.
(20) Bravo-Gutiérrez, O. A.; Falfán-Valencia, R.; Ramírez-Venegas, A.; Sansores, R. H.; Ponciano-Rodríguez, G.; Pérez-Rubio, G. Lung damage caused by heated tobacco products and electronic nicotine delivery systems: a systematic review. *Int. J. Env. Res. Public Health* **2021**, *18* (8), 4079.
(21) Fried, N. D.; Gardner, J. D. Heat-not-burn tobacco products: an emerging threat to cardiovascular health. *American Journal of Physiology-Heart and Circulatory Physiology* **2020**, *319* (6), H1234-H1239.
(22) Zaitsu, M.; Kono, K.; Hosokawa, Y.; Miyamoto, M.; Nanishi, K.; Okawa, S.; Niki, S.; Takahashi, K.; Yoshihara, S.; Kobashi, G. Maternal heated tobacco product use during pregnancy and allergy in offspring. *Allergy* **2023**, *78* (4), 1104-1112.
(23) Rungraungrayabkul, D.; Gaewkhiew, P.; Vichayanrat, T.; Shrestha, B.; Buajeeb, W. What is the impact of nicotine pouches on oral health: a systematic review. *BMC Oral Health* **2024**, *24* (1), 889. DOI: 10.1186/s12903-024-04598-8.
(24) Reimann, H.; Berger, M.; Eckert, E.; Merches, K.; Börnke, F. Beyond smoking: Risk assessment of nicotine in pouches. *Toxicology Reports* **2024**, *13*, 101779. DOI: https://doi.org/10.1016/j.toxrep.2024.101779.
(25) Kent, J. T.; Mok, G.; Austin, E. Nicotine Toxicity From Repeat Use of Nicotine Pouches. *Nicotine and Tobacco Research* **2025**, *27* (4), 767-768.
(26) *Zakon o omejevanju uporabe tobačnih in povezanih izdelkov (ZOUTPI)*. 9/17 (popr. 29/17, 31/24). https://pisrs.si/pregledPredpisa?id=ZAKO6717 (accessed 10.9.2025).

View Article Online
DOI: 10.1039/D6AN00153J

(27) Duren, M.; Atella, L.; Welding, K.; Kennedy, R. D. Nicotine pouches: a summary of regulatory approaches across 67 countries. *Tob. Control* **2024**, *33* (e1), e32-e40.
(28) NIJZ. *Uporaba novih tobačnih in nikotinskih izdelkov med mladimi v Sloveniji narašča*. https://nijz.si/zivljenjski-slog/tobacni-in-povezani-izdelki/uporaba-novih-tobacnih-in-nikotinskih-izdelkov-med-mladimi-v-sloveniji-narasca/ (accessed 11.9.2025).
(29) *Ministrstvo za zdravje (Direktorat za javno zdravje). Kajenje in tobak*. 2024. https://www.gov.si/teme/kajenje-in-tobak/ (accessed 11.12.2025).
(30) Slovenije, V. R. *Strategija za zmanjševanje posledic rabe tobaka ZA SLOVENIJO BREZ TOBAKA 2022-2030*. 2022. https://www.gov.si/assets/ministrstva/MZ/DOKUMENTI/ZDRAVJE/Preventiva-in-skrb-za-zdravje/Strategija-za-Slovenijo-brez-tobaka.pdf (accessed 11.9.2025).
(31) NIJZ. *ZA Slovenijo brez tobaka in nikotina 2040*. 2023. https://nijz.si/zivljenjski-slog/tobacni-in-povezani-izdelki/za-slovenijo-brez-tobaka-in-nikotina-2040/ (accessed 11.9.2025).
(32) Organization, W. H. *WHO study group on tobacco product regulation. Report on the scientific basis of tobacco product regulation: tenth report of a WHO study group*; World Health Organization, 2025.
(33) ASIA, T. *China Regulates Nicotine Pouches*. 2026. https://www.tobaccoasia.com/news/china-regulates-nicotine-pouches/ (accessed 20.3.2026).
(34) *CORESTA Smokeless Tobacco Reference Products*. https://www.coresta.org/coresta-smokeless-tobacco-reference-products (accessed 3.3.2025.
(35) Tran, H.; Tyx, R. E.; Valentin, L.; Mahoney, M.; Stanfill, S.; Watson, C. H. Total and unprotonated (freebase) nicotine content in new types of oral 'tobacco-free'nicotine products. *Tob. Control* **2024**.
(36) Qi, D.; Zhou, Y.; Wang, J.; Fei, T.; Wu, D.; Lu, J. Determination of volatiles in flue-cured tobacco by gas chromatography–mass spectrometry (GC–MS) with chemometrics. *Anal. Lett.* **2022**, *55* (9), 1398-1411.
(37) Merckel, C.; Pragst, F.; Ratzinger, A.; Aebi, B.; Bernhard, W.; Sporkert, F. Application of headspace solid phase microextraction to qualitative and quantitative analysis of tobacco additives in cigarettes. *Journal of Chromatography A* **2006**, *1116* (1-2), 10-19.
(38) Leigh, N. J.; Page, M. K.; Robinson, D. L.; Heldwein, S. D.; O'Connor, R. J.; Goniewicz, M. L. Nicotine, Humectants, and Tobacco-Specific Nitrosamines (TSNAs) in IQOS Heated Tobacco Products (HTPs): A Cross-Country Study. *Toxics* **2024**, *12* (3), 180.
(39) Lawler, T. S.; Stanfill, S. B.; Zhang, L.; Ashley, D. L.; Watson, C. H. Chemical characterization of domestic oral tobacco products: Total nicotine, pH, unprotonated nicotine and tobacco-specific N-nitrosamines. *Food Chem. Toxicol.* **2013**, *57*, 380-386. DOI: https://doi.org/10.1016/j.fct.2013.03.011.
(40) Rantaša, M.; Majer, D.; Finšgar, M. A Review of Analytical Techniques for the Analysis of Oral Smokeless Products and Heated Tobacco Products. *Crit. Rev. Anal. Chem.* **2025**, 1-19.
(41) Stanfill, S.; Tran, H.; Tyx, R.; Fernandez, C.; Zhu, W.; Marynak, K.; King, B.; Valentín-Blasini, L.; Blount, B. C.; Watson, C. Characterization of Total and Unprotonated (Free) Nicotine Content of Nicotine Pouch Products. *Nicotine Tobacco Res.* **2021**, *23* (9), 1590-1596. DOI: 10.1093/ntr/ntab030 (acccessed 11/19/2024).
(42) Patel, P. H.; Conrad, K. L.; Pathiranage, A. L.; Hiatt, L. A. Practical Applications of Organic Chemistry: Improving Student Knowledge of GC–MS through Qualitative Analysis of Nicotine in Electronic Cigarette Vapors. *Journal of Chemical Education* **2020**, *97* (11), 4117-4122. DOI: 10.1021/acs.jchemed.0c00179.
(43) Gómez-Siurana, A.; Marcilla, A.; Beltrán, M.; Berenguer, D.; Martínez-Castellanos, I.; Menargues, S. TGA/FTIR study of tobacco and glycerol–tobacco mixtures. *Thermochim. Acta* **2013**, *573*, 146-157. DOI: https://doi.org/10.1016/j.tca.2013.09.007.
(44) Zhang, H.; Yang, J.; Zhu, F.; Luo, C.; Pang, Y.; Zhu, B.; Luo, Y.; Li, X.; Jiang, X.; Li, D. Headspace Solid-Phase Microextraction Coupled with Gas Chromatography–Mass Spectrometry (HS-SPME-GC–MS) for the Characterization of Cigar Leaves. **2021**.
(45) Hayes, E. *How HEETS are made: Production process*. IQOS Abu Dhabi, 2024. https://iqos-abudhabi.ae/how-heets-are-made-production-process/ (accessed 24.10.2025).

(46) Kim, Y.-H.; Kim, S.-H. Development and validation of a method for preparing heated tobacco product aerosol condensate (HTPAC) for large-scale toxicity data acquisition. *Ecotoxicology and Environmental Safety* **2023**, *267*, 115621. DOI: https://doi.org/10.1016/j.ecoenv.2023.115621.
(47) Banožić, M.; Aladić, K.; Jerković, I.; Jokić, S. Volatile organic compounds of tobacco leaves versus waste (scrap, dust, and midrib): extraction and optimization. *Journal of the Science of Food and Agriculture* **2021**, *101* (5), 1822-1832.
(48) Shi, J.; Yang, Y.; Zhang, T.; Liang, K.; Guo, L.; Deng, R.; Liu, K.; Ren, Y. Multiple analyses of main flavor components in reconstituted tobacco and transfer behavior of their key substances during heating. *J. Sep. Sci.* **2024**, *47* (14), 2400250.
(49) Liu, T.; Kong, N.; Cao, J.; Chen, G.; Deng, Z.; Wen, L.; Zhang, Z.; Guo, C.; Wu, R.; Yang, Y. Transcriptomic and metabolomic analysis of carotenoid metabolism and accumulation of carotenoid-derived products in tobacco leaves before and after curing. *Frontiers in Plant Science* **2025**, *16*, 1671379.
(50) Son, Y.; Wackowski, O.; Weisel, C.; Schwander, S.; Mainelis, G.; Delnevo, C.; Meng, Q. Evaluation of E-Vapor Nicotine and Nicotyrine Concentrations under Various E-Liquid Compositions, Device Settings, and Vaping Topographies. *Chem. Res. Toxicol.* **2018**, *31* (9), 861-868. DOI: 10.1021/acs.chemrestox.8b00063.
(51) Czoli, C. D.; Goniewicz, M. L.; Palumbo, M.; Leigh, N.; White, C. M.; Hammond, D. Identification of flavouring chemicals and potential toxicants in e-cigarette products in Ontario, Canada. *Can. J. Public Health* **2019**, *110* (5), 542-550.
(52) *Consolidated text: Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006 (Text with EEA relevance)*. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A02008R1272-20250901 (accessed 28.11.2025).
(53) Kim, Y. W.; Kim, M. J.; Chung, B. Y.; Bang, D. Y.; Lim, S. K.; Choi, S. M.; Lim, D. S.; Cho, M. C.; Yoon, K.; Kim, H. S. Safety evaluation and risk assessment of d-limonene. *Journal of Toxicology and Environmental Health, Part B* **2013**, *16* (1), 17-38.
(54) Sachdeva, J.; Karunananthan, A.; Shi, J.; Dai, W.; Kleinman, M. T.; Herman, D.; Kloner, R. A. Flavoring Agents in E-cigarette Liquids: A Comprehensive Analysis of Multiple Health Risks. *Cureus* **2023**, *15* (11).
(55) Cartus, A. T.; Lachenmeier, D. W.; Guth, S.; Roth, A.; Baum, M.; Diel, P.; Eisenbrand, G.; Engeli, B.; Hellwig, M.; Humpf, H. U. Acetaldehyde as a food flavoring substance: aspects of risk assessment. *Mol. Nutr. Food Res.* **2023**, *67* (23), 2200661.
(56) Catalano, A.; Mariconda, A.; D'Amato, A.; Iacopetta, D.; Ceramella, J.; Marra, M.; Saturnino, C.; Sinicropi, M. S.; Longo, P. Aldehydes: What We Should Know About Them. *Organics* **2024**, *5* (4), 395-428.
(57) Wu, Z.; Tan, B.; Liu, Y.; Dunn, J.; Martorell Guerola, P.; Tortajada, M.; Cao, Z.; Ji, P. Chemical Composition and Antioxidant Properties of Essential Oils from Peppermint, Native Spearmint and Scotch Spearmint. *Molecules* **2019**, *24* (15), 2825.
(58) Zheljazkov, V. D.; Cantrell, C. L.; Astatkie, T.; Hristov, A. Yield, content, and composition of peppermint and spearmints as a function of harvesting time and drying. *Journal of Agricultural and Food Chemistry* **2010**, *58* (21), 11400-11407.
(59) Mallock-Ohnesorg, N.; Rinaldi, S.; Malke, S.; Dreiack, N.; Pieper, E.; Laux, P.; Schulz, T.; Zimmermann, R.; Luch, A. Oral nicotine pouches with an aftertaste? Part 1: screening and initial toxicological assessment of flavorings and other ingredients. *Archives of Toxicology* **2023**, *97* (9), 2357-2369. DOI: 10.1007/s00204-023-03538-9.
(60) EU. *REGULATION (EC) No 1334/2008 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 December 2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods and amending Council Regulation (EEC) No 1601/91, Regulations (EC) No 2232/96 and*

(EC) No 110/2008 and Directive 2000/13/EC. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A02008R1334-20250625 (accessed 29.10.2025).
(61) Kheawfu, K.; Kaewpinta, A.; Chanmahasathien, W.; Rachtanapun, P.; Jantrawut, P. Extraction of nicotine from tobacco leaves and development of fast dissolving nicotine extract film. *Membranes* **2021**, *11* (6), 403.
(62) Omaiye, E. E.; Luo, W.; McWhirter, K. J.; Pankow, J. F.; Talbot, P. Disposable Puff Bar Electronic Cigarettes: Chemical Composition and Toxicity of E-liquids and a Synthetic Coolant. *Chem. Res. Toxicol.* **2022**, *35* (8), 1344-1358. DOI: 10.1021/acs.chemrestox.1c00423 From NLM.
(63) Page, M. K.; Leigh, N. J.; Block, A., C; Marrano, P. H.; Travers, M. B.; Adrover, J. E.; Maley, G. E.; Koenig, L. A.; Salem, E. M.; Heldwein, S. D.; et al. Comparison of Nicotine and Selected Flavoring Contents Between Tobacco-Free and Tobacco-Containing Oral Pouches. *Nicotine Tobacco Res.* **2025**. DOI: 10.1093/ntr/ntaf105 (acccessed 10/30/2025).
(64) Shaikh, S. B.; Tung, W. C.; Pang, C.; Lucas, J.; Li, D.; Rahman, I. Flavor Classification/Categorization and Differential Toxicity of Oral Nicotine Pouches (ONPs) in Oral Gingival Epithelial Cells and Bronchial Epithelial Cells. *Toxics* **2022**, *10* (11), 660.
(65) Miller-Holt, J.; Baskerville-Abraham, I.; Sakimura, M.; Fukushima, T.; Puglisi, A.; Gafner, J. In vitro evaluation of mutagenic, cytotoxic, genotoxic and oral irritation potential of nicotine pouch products. *Toxicology Reports* **2022**, *9*, 1316-1324. DOI: https://doi.org/10.1016/j.toxrep.2022.06.008.
(66) Guideline, I. H. Validation of analytical procedures Q2 (R2). *ICH: Geneva, Switzerland* **2022**.
(67) Majer, D.; Mastnak, T.; Finšgar, M. An Advanced Statistical Approach Using Weighted Linear Regression in Electroanalytical Method Development for Epinephrine, Uric Acid and Ascorbic Acid Determination. *Sensors* **2020**, *20* (24), 7056.
(68) Bekki, K.; Inaba, Y.; Uchiyama, S.; Kunugita, N. Comparison of chemicals in mainstream smoke in heat-not-burn tobacco and combustion cigarettes. *Journal of UOEH* **2017**, *39* (3), 201-207.
(69) *Directive 2014/40/EU of the European Parliament and of the Council of 3 April 2014 on the approximation of the laws, regulations and administrative provisions of the Member States concerning the manufacture, presentation and sale of tobacco and related products and repealing Directive 2001/37/EC*. 2014. https://eur-lex.europa.eu/eli/dir/2014/40/oj/eng (accessed 5.11.2025).
(70) FDA. *FDA Proposes Significant Step Toward Reducing Nicotin to Minimally or Nonaddictive Level in Cigarettes and Certain Other Combusted Tobacco Products*. 2025. https://www.fda.gov/news-events/press-announcements/fda-proposes-significant-step-toward-reducing-nicotine-minimally-or-nonaddictive-level-cigarettes (accessed 17.3.2026).
(71) Djordjevic, M. V.; Doran, K. A. Nicotine Content and Delivery Across Tobacco Products. In *Nicotine Psychopharmacology*, Henningfield, J. E., London, E. D., Pogun, S. Eds.; Springer Berlin Heidelberg, 2009; pp 61-82.
(72) Han, S.; Li, X.; Cui, L.; Fang, Q.; Fu, Y.; Feng, P.; Tian, Y.; Sun, J.; Wang, Y.; Song, W. Comprehensive chemical characterization of nicotine pouches. *Microchem. J.* **2025**, 116196.
(73) *141 DECREE of 16 May 2023 on tobacco-free nicotine pouches*. https://technical-regulation-information-system.ec.europa.eu/en/notification/16379/text/F/EN (accessed 3.11.2025).
(74) Mallock, N.; Schulz, T.; Malke, S.; Dreiack, N.; Laux, P.; Luch, A. Levels of nicotine and tobacco-specific nitrosamines in oral nicotine pouches. *Tob. Control* **2024**, *33* (2), 193-199.
(75) FDA. *Nicotine Pouch Products Authorized by the FDA*. 2025. https://www.fda.gov/tobacco-products/market-and-distribute-tobacco-product/nicotine-pouch-products-authorized-fda (accessed 17.3.2025).
(76) Golia, E.; Dimirkou, A.; Mitsios, I. Heavy-metal concentration in tobacco leaves in relation to their available soil fractions. *Commun. Soil Sci. Plant Anal.* **2009**, *40* (1-6), 106-120.
(77) Katakojwala, R.; Mohan, S. V. Microcrystalline cellulose production from sugarcane bagasse: Sustainable process development and life cycle assessment. *Journal of Cleaner Production* **2020**, *249*, 119342. DOI: https://doi.org/10.1016/j.jclepro.2019.119342.
(78) Regassa, G.; Chandravanshi, B. S. Levels of heavy metals in the raw and processed Ethiopian tobacco leaves. *SpringerPlus* **2016**, *5* (1), 232. DOI: 10.1186/s40064-016-1770-z.

(79) Back, S.; Masser, A. E.; Rutqvist, L. E.; Lindholm, J. Harmful and potentially harmful constituents (HPHCs) in two novel nicotine pouch products in comparison with regular smokeless tobacco products and pharmaceutical nicotine replacement therapy products (NRTs). *BMC Chemistry* **2023**, *17* (1), 9. DOI: 10.1186/s13065-023-00918-1.
(80) Azzopardi, D.; Liu, C.; Murphy, J. Chemical characterization of tobacco-free "modern" oral nicotine pouches and their position on the toxicant and risk continuums. *Drug Chem Toxicol* **2022**, *45* (5), 2246-2254.
(81) Jablonski, J. J.; Cheetham, A. G.; Martin, A. M. Market Survey of Modern Oral Nicotine Products: Determination of Select HPHCs and Comparison to Traditional Smokeless Tobacco Products. *Separations* **2022**, *9* (3), 65.
(82) Granata, S.; Vivarelli, F.; Morosini, C.; Canistro, D.; Paolini, M.; Fairclough, L. C. Toxicological Aspects Associated with Consumption from Electronic Nicotine Delivery System (ENDS): Focus on Heavy Metals Exposure and Cancer Risk. *Int. J. Mol. Sci.* **2024**, *25* (5), 2737. DOI: https://doi.org/10.3390/ijms25052737 Publicly Available Content Database.
(83) Koutela, N.; Fernández, E.; Saru, M.-L.; Psillakis, E. A comprehensive study on the leaching of metals from heated tobacco sticks and cigarettes in water and natural waters. *Science of The Total Environment* **2020**, *714*, 136700. DOI: https://doi.org/10.1016/j.scitotenv.2020.136700.
(84) Desideri, D.; Roselli, C.; Fagiolino, I.; Meli, M. A. Toxic elements in human saliva of smokeless tobacco users. *J. Anal. Toxicol.* **2018**, *42* (6), 417-424.
(85) Rubio Armendáriz, C.; Garcia, T.; Soler, A.; Gutiérrez Fernández, Á. J.; Glez-Weller, D.; Luis González, G.; de la Torre, A. H.; Revert Gironés, C. Heavy metals in cigarettes for sale in Spain. *Environmental Research* **2015**, *143*, 162-169. DOI: https://doi.org/10.1016/j.envres.2015.10.003.
(86) Viana, G. F. d. S.; Garcia, K. S.; Menezes-Filho, J. A. Assessment of carcinogenic heavy metal levels in Brazilian cigarettes. *Environ Monit Assess* **2011**, *181* (1), 255-265. DOI: 10.1007/s10661-010-1827-3.
(87) Gómez-Siurana, A.; Marcilla, A.; Beltran, M.; Martinez, I.; Berenguer, D.; García-Martínez, R.; Hernández-Selva, T. Thermogravimetric study of the pyrolysis of tobacco and several ingredients used in the fabrication of commercial cigarettes: Effect of the presence of MCM-41. *Thermochim. Acta* **2011**, *523* (1), 161-169. DOI: https://doi.org/10.1016/j.tca.2011.05.018.
(88) Sung, Y. J.; Seo, Y. B. Thermogravimetric study on stem biomass of Nicotiana tabacum. *Thermochim. Acta* **2009**, *486* (1), 1-4. DOI: https://doi.org/10.1016/j.tca.2008.12.010.
(89) Marcilla, A.; Beltran, M. I.; Gómez-Siurana, A.; Martinez-Castellanos, I.; Berenguer, D.; Pastor, V.; García, A. N. TGA/FTIR study of the pyrolysis of diammonium hydrogen phosphate–tobacco mixtures. *J. Anal. Appl. Pyrolysis* **2015**, *112*, 48-55. DOI: https://doi.org/10.1016/j.jaap.2015.02.023.
(90) Marcilla, A.; Berenguer, D. TGA/FTIR study of the decomposition of Heet tobacco in presence of zeolites and silicate compounds. *Materials Science and Engineering: B* **2023**, *295*, 116593. DOI: https://doi.org/10.1016/j.mseb.2023.116593.
(91) Fairouz Saad, S.; Berbar, Y.; Amara, M. Grafted Amberlite 200C Resin for Enhanced Salicylic Acid Adsorption. *Journal of Water Chemistry and Technology* **2024**, *46* (6), 543-553. DOI: 10.3103/S1063455X24060110.
(92) Agioutanti, E.; Keles, C.; Sarver, E. A thermogravimetric analysis application to determine coal, carbonate, and non-carbonate minerals mass fractions in respirable mine dust. *Journal of Occupational and Environmental Hygiene* **2020**, *17* (2-3), 47-58.
(93) Polat, S.; Apaydin-Varol, E.; Pütün, A. E. Thermal decomposition behavior of tobacco stem Part I: TGA–FTIR–MS analysis. *Energy Sources, Part A: Recovery, Utilization, and Environmental Effects* **2016**, *38* (20), 3065-3072. DOI: 10.1080/15567036.2015.1129373.

Data Availability Statement

View Article Online
DOI: 10.1039/D6AN00153J

The data supporting this study's findings are available upon request.