


Real-Time Chemical Analysis of E-Cigarette Aerosols by Means of Secondary Electrospray Ionization Mass Spectrometry

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**REAL-TIME CHEMICAL ANALYSIS OF E-CIGARETTE
AEROSOLS BY MEANS OF SECONDARY ELECTROSPRAY
IONIZATION – MASS SPECTROMETRY**

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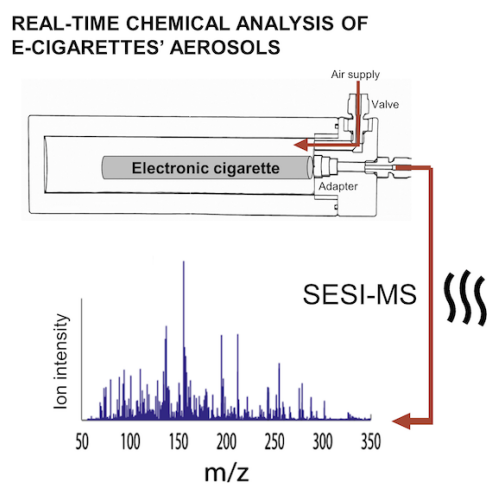
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ABSTRACT

Chemicals analysis of aerosols collected from electronic cigarettes has shown that these devices produce vapors that contain harmful and potentially harmful compounds. Conventional analytical methods used for the analysis of electronic cigarettes do not reflect the actual composition of the aerosols generated since they usually neglect the changes in chemical composition that occur during the aerosol generation process and after collection. The aim of this work was to develop and apply a method for real-time analysis of electronic cigarette aerosols, based on secondary electrospray ionization coupled to high-resolution mass spectrometry, by mimicking the “vaping” process. Electronic cigarette aerosols were successfully analyzed and quantitative differences were found between liquids and aerosols. Thanks to the high sensitivity shown by this method, more than 250 chemical substances were detected in aerosols, some of them showing a high correlation with the operating power of electronic cigarettes. The method also allows proper quantification of several chemical components such as alkaloids and flavor compounds.

Graphical Abstract



INTRODUCTION

Electronic cigarettes (ECs) or “electronic nicotine delivery systems” are battery-powered products enjoying great popularity. World wide web searches for ‘electronic cigarettes’ (ECs) have increased several fold in recent years and now surpass searches for nicotine medications.^[1] International studies show that approximately 8% of adult smokers have tried ECs and that they are rapidly gaining acceptance as an alternative to conventional tobacco cigarettes.^[2a,b] By January 2014 there were 466 brands (each with its own website) and 7764 unique flavours.^[3] It is estimated that there is a net increase of 10.5 brands and 242 new flavors per month.^[3]

ECs usually operate on the following principle: a battery is connected to an atomizer (heating element) that vaporizes EC-liquid, which exits the EC as an aerosol via a cartridge (mouthpiece). The liquid that is vaporized is a solution of water, propylene glycol, glycerin, and optionally nicotine and flavorings. Nicotine concentrations can range from 0 to 18 mg/mL.

Despite their popularity, there are relatively few studies on ingredients of EC liquids^[4a,b] and even fewer about the composition of EC aerosol.^[5a,b,c,d] Studies have shown that EC-liquids contain nicotine impurities,^[4,6] possibly metal and silicate particles^[7] and certain toxicants that have been attributed to flavors.^[8] Recent research suggests that there are uncertainties about EC-liquid ingredients that might be chemically altered during the vaporization process and that levels of carbonyls rapidly increase with increased battery voltage.^[5] Almost nothing is known about the effects of EC-vapor during long-term exposure, prenatal development, or on adult health.

Therefore, one of the key points to establish a proper control for ECs is to determinate their chemical composition.^[9] It is obvious that the presence (or absence) of harmful and potentially harmful compounds such as aldehydes,^[10] tobacco-specific

nitrosamines,^[11] and volatile organic compounds (VOCs)^[12] should play an important role for the decision-making process in public health agencies.^[13] In this field, EC manufactures provide little or no information about the chemical compounds that are added to EC-liquids, apart from nicotine, whose content has been proven by different studies to not match what labels claim.^[6,14a,b]

A few authors have studied the chemical composition of ECs by evaluating the EC-liquids^[4,15a,b] and/or the aerosols generated.^[5,6,16,a,b] 29 relevant articles were recently reviewed by Cheng,^[9] who concluded that scientifically validated aerosol generation methods are needed to obtain reliable measures of chemical quantities. In this sense, the analytical method most widely used is liquid chromatography,^[4,5,9,15-17] with spectroscopic or mass spectrometric detection. Direct injection of e-liquids into the chromatographic system can be done, but it has been argued that this neglects the changes in chemical composition that occur during the aerosol generation process.^[18] Gas chromatography^[9,12,15,16,19] has also been proposed as a way of analyzing the vapors generated, although it is only suitable for volatile and thermally stable compounds. Finally, in order to circumvent these problems, some authors^[5,6,16] have designed smoking devices that allow collection of aerosols for further analysis. However, these offline approaches significantly extend the analysis time and do not really assure a proper analysis of the aerosols generated: some compounds could react with the solvent, and volatile compounds could be lost.

Secondary electrospray ionization (SESI) is an ambient MS technique used for the analysis of vapors and aerosols in real time.^[20a,b] SESI has been recently proposed for the analysis of explosives,^[21] illicit drugs,^[22] volatile compounds from bacterial cultures^[23] and exhaled human breath.^[24] Even though it has not been tried before, SESI may be a truly appropriate technique for the analysis of EC aerosols since they

could be directly sampled into the SESI source, allowing a direct real-time analysis that mimics the real “vaping” process. The aim of this work was to develop and apply, by means of SESI, a method for the real-time analysis of EC aerosols, in an attempt to solve the problems currently associated with their analysis.

RESULTS & DISCUSSION

Analysis of EC aerosols by real-time SESI

It has been argued that the analysis of EC-liquids by liquid chromatography-mass spectrometry (LC-MS) techniques is not representative for the sample composition since the process of aerosol generation may result in changes in chemical composition.^[5,18] One way of circumventing this problem is to use an analytical method that allows the direct analysis of aerosols generated by ECs. Such a method should be based on a state-of-the art technique for the real-time analysis of vapors. Among them, SESI coupled to high-resolution mass spectrometry (HRMS) has been proven to be a robust and efficient methodology for the analysis of a broad range of gaseous and aerosol samples.^[20,25] Therefore, to show the capabilities of SESI-HRMS for analyzing EC aerosols (an approach that, to the best of our knowledge, has never been tried before) different EC and EC-liquids were analyzed as described in the methods section. Figure 1 shows two examples of spectra obtained and their comparison with data obtained by direct analysis of the EC-liquids by ESI-HRMS – a more usual approach that delivers results that are close to those obtained by LC-MS. As can be seen, rich spectra were obtained, with the main peaks corresponding to nicotine. Comparison with spectra obtained by ESI-HRMS shows similar results, with the main peaks present in both spectra. Fragmentation was found to be negligible, as expected for these soft ionization techniques. However, SESI-HRMS and ESI-HRMS

spectra are clearly quantitatively different. While solvent peaks (e.g. from PG) show high intensities in ESI spectra, their intensities in SESI spectra are almost negligible. On the other hand, some peaks are strongly enhanced in SESI mass spectra, such as pyrazine-C3 and pyrazine-C4.

This finding supports the concerns raised by some authors that the chemical composition of EC aerosols may differ from the composition of EC-liquids (at least quantitatively) and that direct analysis of EC-liquids may therefore not be appropriate for assessing exposure to EC-related hazardous compounds. These differences may be based on the volatility of the EC-liquid components and may be even bigger when comparing with offline techniques (e.g. LC-MS) that may result in a more significant loss of volatile compounds. Compounds with higher volatilities, such as pyrazines, are enhanced in the process of generation of the aerosol while compounds with lower volatilities, such as PG, are decreased.

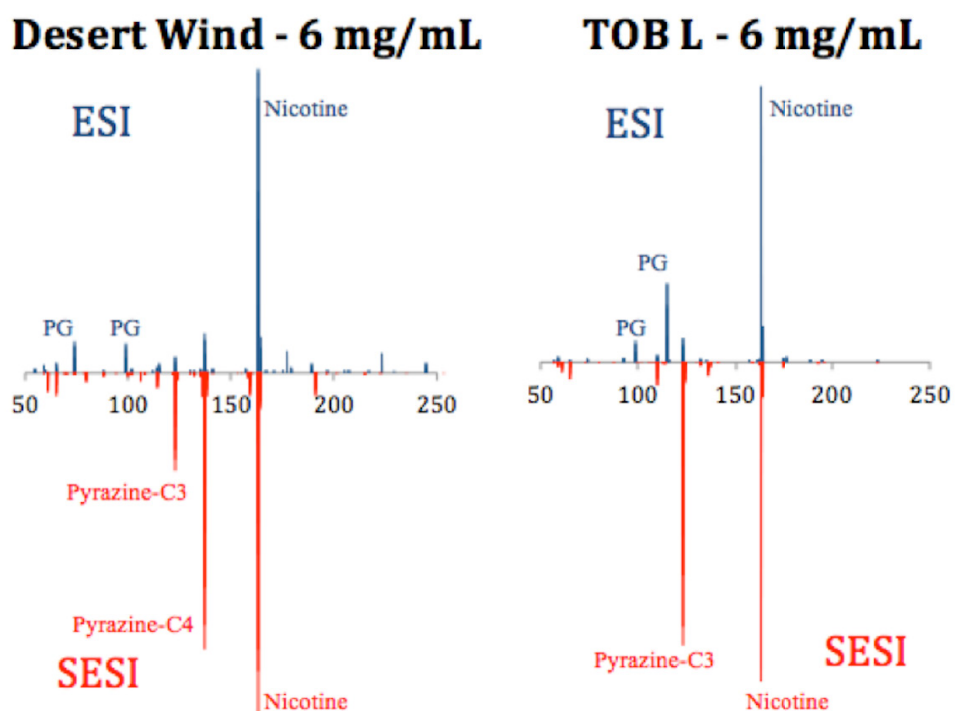


Figure 1. High-resolution mass spectra from two e-liquids analyzed by ESI (blue) and Secondary ESI (red, EC: EVOD2). EC: electronic cigarette; ESI: electrospray ionization

Quantification of nicotine

One of the main disadvantages of SESI-HRMS for analysis of gaseous samples is the difficulty to achieve a proper quantification, mainly because it is not easy to obtain standard gas samples of known concentration. The SESI smoking device can solve this problem, since it can be used to generate aerosols from standard liquid samples in the same way as it is used to generate aerosols from EC. To prove this concept, a calibration curve (ordinary least squares) was built using nicotine standards with the EC EVOD2. A good linear relation was found for signal intensities versus nicotine concentration (Figure 2) from 5 to 20 mg/mL. On the basis of this calibration curve, nicotine contents of all EC-liquids studied were tested (Figure 2). Results showed good agreement with the contents claimed by the manufacturers, including those EC-liquids labeled as nicotine-free, with the exception of the “Happy Liquid” brand, which had nicotine concentrations that were about twice those claimed on the labels.

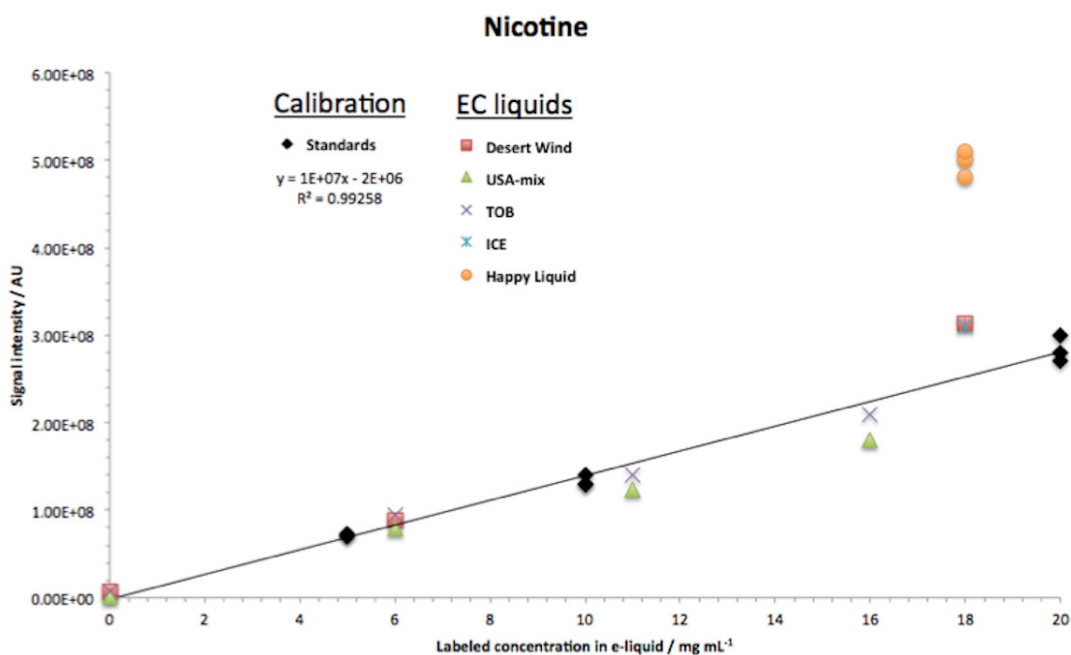


Figure 2. Calibration curve and quantification of nicotine in several e-liquids (e-cig: EVOD2) by SESI-HRMS

Occurrence and quantification of other chemical compounds

In addition to nicotine, a range of compounds has been found by different authors in EC aerosols and EC-liquids.^[9] This includes some harmful or potentially harmful substances such as aldehydes, tobacco alkaloids, tobacco-specific nitrosamines (TSNAs), polycyclic aromatic hydrocarbons (PAHs) and volatile organic compounds (VOCs). Supposed harmless substances such as different flavors and solvents complete the list. In order to check the presence of these compounds in EC aerosols by means of SESI-HRMS, their signals were extracted from the SESI mass spectra. To achieve this, m/z values of protonated compounds ($M+H^+$) were calculated and a search was run in the EC aerosols spectra, which were recorded from 50 to 500 u, within a mass accuracy window of ± 2 ppm. All compounds putatively identified in the EC aerosols and EC-liquids studied are listed in Table S2 and Figure 3 shows the signals obtained from some of these compounds in 2-3 consecutive puffs. Of

particular interest is the occurrence of several tobacco alkaloids, most likely co-extracted during nicotine isolation from tobacco, and also the presence of several harmful VOCs, especially benzene and toluene. In contrast, no aldehydes, TSNAs or PAHs were found. It has been recently found^[18] that aldehydes are only emitted due to thermal decomposition of liquid components at high-power levels ('dry puffs') that are usually avoided by the consumers. However, it should be highlighted that the two aldehydes mainly found in EC, formaldehyde and acetaldehyde, are below the mass range of the instrument and could not be tested. TSNAs, one of the more important groups of carcinogens in tobacco products, are produced during the curing and processing of tobacco leaves; therefore, their absence in EC aerosols is expected and has been cited as a major advantage when comparing tobacco and EC toxicity. A similar argument can be made for the absence of PAHs in EC aerosols since they are produced during incomplete combustion of tobacco. However, it should be stated that the great sensitivity usually achieved by SESI, with limits of detection in the low ppt_v range, does not apply for PAHs because they are not easily ionized by ESI.^[26] Therefore, it may be possible that limits of detection for these compounds are higher than usual e-cig concentrations.^[9] Finally, the occurrence of a large range of flavors and aroma esters should be highlighted.

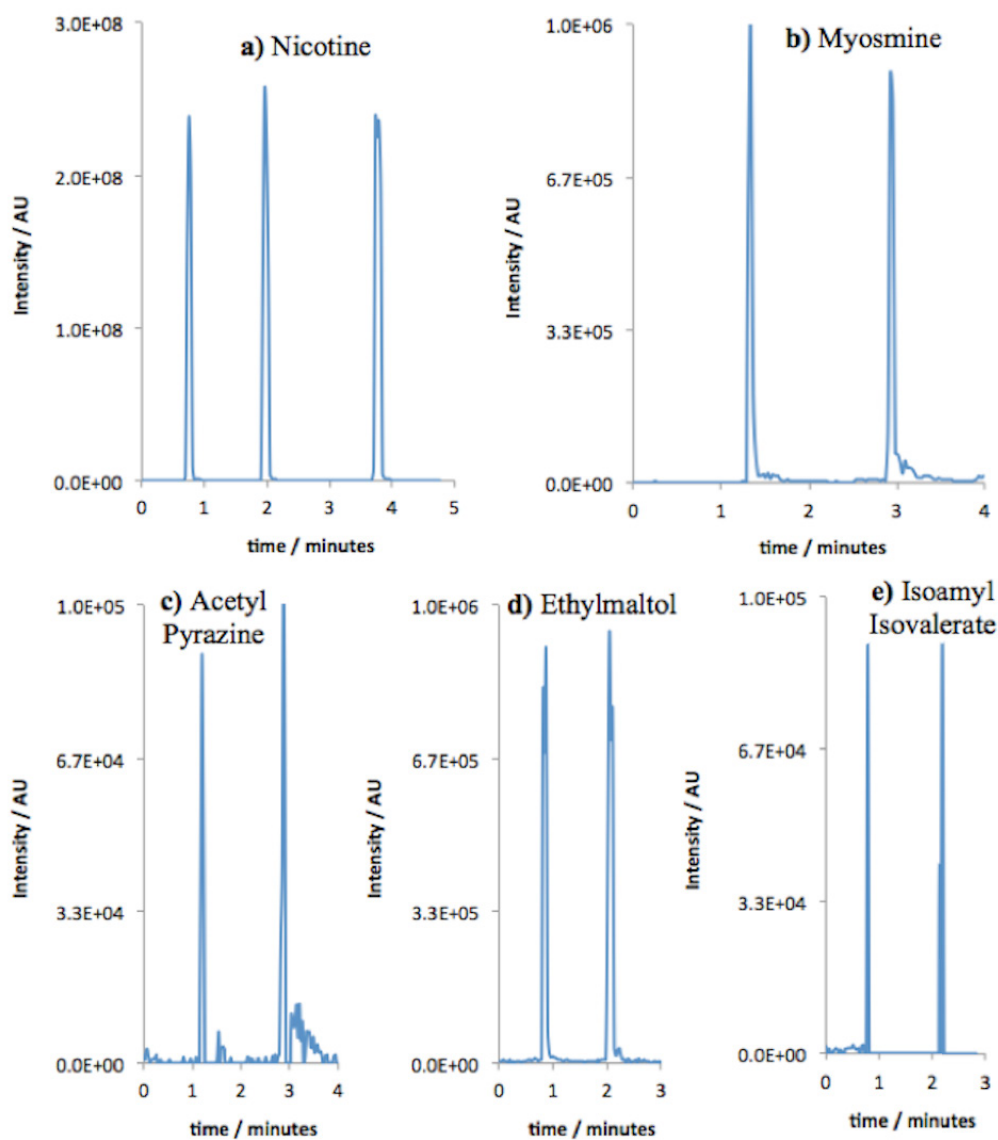


Figure 3. Selected ion signals (mass window: ± 2 ppm) obtained by SESI-HRMS from a) EVOD2, TOB H, 3 puffs; m/z: 163.1230. b) EVOD2, DW 18, 2 puffs; m/z: 147.0917. c) EVOD2, DW 0, 2 puffs; m/z: 123.0553 d) EVOD2, USA-Mix W, 2 puffs; m/z: 141.0546. e) EVOD2, HL Apfel, 2 puffs; m/z: 173.1536. SESI: Secondary electrospray ionization; HRMS: high-resolution mass spectrometry

Some of the compounds found were selected for quantification, with the aim to cover different chemical families. In this way, standards of known concentration of myosmine, tetramethylpyrazine, acetylpyrazine, ethylmaltol, isoamyl isovalerate and

ethyl isovalerate were analyzed as EC-liquids, and their signal intensities were used for calibration (ordinary least squares). The results obtained in the analysis of all the EC-liquids studied in this work are shown in Table 1. Linear responses were found from 0.001 to 5 mg/mL for pyrazines, ethylmaltol and fruity esters and from 0.1 to 50 mg/L for myosmine.

Table 1. Quantification of selected compounds in EC aerosols (EVOD 2) by SESI-HRMS, based on calibration curves using standards.

E-LIQUID	Alkaloids		Smoke smell	Roast smell	Sweet smell	Fruity esters	
	Myosmine <i>mg/L</i>	% Nic	Pyrazine-C4 <i>mg/mL</i>	Pyrazine-acetyl <i>mg/mL</i>	Ethylmaltol <i>mg/mL</i>	Isoamyl isovalerate <i>mg/mL</i>	Ethyl isovalerate <i>mg/mL</i>
DW0	-	-	2.4±0.2	0.05±0.003	0.07±0.005	-	-
DW6	13±1	0.22	1.9±0.2	0.05±0.003	0.11±0.009	-	-
DW18	39±3	0.22	1.2±0.1	0.03±0.002	0.12±0.01	-	-
ICE	44±3	0.24	-	0.02±0.002	-	-	-
Usa Mix W	-	-	0.44±0.03	0.19±0.02	0.38±0.03	-	-
USA mix G	4±0.5	0.07	0.13±0.01	0.11±0.01	0.26±0.03	-	-
USA mix B	6±0.8	0.06	0.27±0.02	0.47±0.05	0.48±0.04	-	-
USA mix R	11±1	0.07	0.12±0.01	0.79±0.06	0.61±0.05	-	-
TOB Z	-	-	0.01±0.002	0.07±0.004	0.40±0.03	-	-
TOB L	1±0.2	0.02	0.01±0.002	0.09±0.005	0.42±0.04	-	-
TOB M	2±0.3	0.02	0.01±0.002	0.21±0.02	0.11±0.009	-	-
TOB H	4±0.5	0.02	0.01±0.002	0.21±0.02	0.21±0.02	-	-
HL Apfel	14±1	0.08	0.01±0.002	-	0.03±0.002	0.21±0.01	0.41±0.02
HL Kirsche	9±1	0.05	0.01±0.002	0.06±0.004	0.04±0.003	-	0.16±0.01
HL Erdbeer	10±1	0.06	0.05±0.004	0.03±0.002	0.06±0.003	-	0.22±0.01

Concentrations of the tobacco alkaloid myosmine in aerosol produced from commercial EC liquids ranged from 1 mg/L to 44 mg/L. It should be noted that myosmine concentrations are strongly correlated to nicotine concentrations and that they remain constant throughout a brand when calculated as percentages of nicotine, with values ranging from 0.02 % for Joyetech TOB to 0.22 % for Collins' EC-liquids.

This is in good agreement with findings by Etter et al.^[4] A large variation in compounds added as flavors (smoke, roast and sweet) was found between different brands with concentration differences, by up to two orders of magnitude. Finally, fruity esters were only found in samples labeled as “fruity flavored” (e.g., apple, cherry and strawberry).

Untargeted analysis

Even though more than 50 compounds, including alkaloids, VOCs and flavors, were detected with the targeted approach, the number of unknown chemical components in EC aerosols is expected to be much higher. To check this, an untargeted analysis, designed to detect any m/z features with a signal intensity higher than 10^5 counts, was performed in positive ionization mode. 142 compounds were detected (Table S3) and, thanks to the high mass accuracy provided by the high-resolution mass spectrometer, a unique chemical formula was assigned to each compound (based on the “seven golden rules” proposed by Kind and Fiehn).^[27] Although several main peaks (up to 57, 40%) correspond to known compounds such as nicotine, trimethylpyrazine and pyridine, the remaining peaks are unidentified compounds whose origin and effect are unknown. The high occurrence of nitrogenated compounds (70%) is notable, especially among major compounds (22 out of the 25 highest intensity features). This fact may be related with the presence of nicotine, its derivatives, and nitrogenated flavors such as pyrazine compounds.

Study of different e-cigarettes

It has been stated that the chemical composition of EC aerosols from the same EC-liquids may vary significantly when changing EC brand.^[5] In this sense, battery

output has been identified as a major factor, with levels of some compounds rapidly increasing from 4 to over 200 times when voltage is changed from 3.2 V to 4.8 V.^[5] To check this, three EC-liquids (Desert Wind 6, USA-mix G and TOB L) were analyzed with three different widely used EC brands (Table S1) ranging from 5 Watts to 35 Watts of power. *m/z* features and their intensities were extracted from the spectra and used to build a partial least square (PLS) regression (Figure S2). As can be seen, the concentration of some compounds in EC aerosols is strongly correlated with the power applied to the EC-liquid by the EC. Among these compounds, the presence of nicotine and pyrazine derivatives should be highlighted, with an increase of 40 and 50 times respectively, when changing the power from 5 Watts to 35 Watts. It should be noticed that PLS regression relies mainly on volatile compounds ($C_5H_4O_4$ and several pyrazine derivatives), which highlights again the significant differences that may exist between aerosol and liquid concentrations based on volatility. This correlation between concentration and power has been previously proposed by Kosminder et al. for carbonyl compounds,^[5] and it seems to be an important factor to be considered when assessing EC-smokers' exposure to these compounds.

Study strengths and limitations

The strengths of our study include the application of a novel method to investigate compounds generated by ECs and that it realistically mimics the whole process of EC-vaping. With this technique, we were able to reproduce findings from other techniques (e.g. ESI) or studies (e.g. power dependence) in addition to revealing new compounds in EC vapor, which may contain critical information for assessing the impact of EC on health. Despite identifying a new set of compounds in EC vapor, we could not assign all features found in EC-vapor to a unique chemical compound,

which is a limitation of this study. Future studies using SESI-MS are needed to evaluate a larger set of EC-brands and liquids to gain more information about the variety of products on the market.

Concluding remarks

A new analytical method for the analysis of EC aerosols, based on a SESI-HRMS smoking device, has been developed. This method allows the real-time analysis of EC aerosols whose composition was found to be different from that of the EC-liquids. In addition, the method allows accurate quantification of chemical components as has been proven for several different compounds such as nicotine and related alkaloids, pyrazine derivatives, ethylmaltol, and fruity esters. Furthermore, it has been shown that more than 250 compounds can be detected in EC aerosols by means of this technique, with the aerosol concentration for some compounds being highly correlated with the operating power of the EC.

EXPERIMENTAL SECTION

Sources of ECs and refill fluids

The most frequently used EC-brands and models vary by country. Therefore, we identified the most popular EC-brands and models according to an international questionnaire covering the American and European markets.^[25] Accordingly, the most frequently used models (sold under various brand names) were the *510* and the *eGo* accounting for >50% of daily EC-users.^[25] Thus the brands and models chosen for this study are among those that dominate the market. The following EC-models were purchased via the internet in February 2015: (i) *EVOD 2* (Kangertech, Shenzhen, China), (ii) *eGo ONE* (Joyetech Electronics, Shenzhen, China), and (iii) *Joye510CC*

(Joyetech Electronics, Shenzhen, China). Additionally, representative liquids (flavored and unflavored) with nicotine concentrations ranging from 0 to 18 mg/mL were obtained. Purchases (table S1) from vendors were conducted under a private name without revealing any affiliation with an institute.

Laboratory procedures

Development of a real-time SESI smoking device

The inlet of a previously developed low-flow secondary electrospray source (LF-SESI, Figure S1)^[28] was modified to accommodate a smoking device (Figure 4 and interactive PDF file in Supporting Information). This device was made from two different plastic pieces: a holder for connecting EC of different diameters that also works as a connector to the LF-SESI source, and a cylinder that encloses the EC and allows an overpressure to be applied by means of an air entrance that simulates the “vaping” process. Synthetic air at 25°C and at a flow rate of 0.2 L/min was used for this purpose.

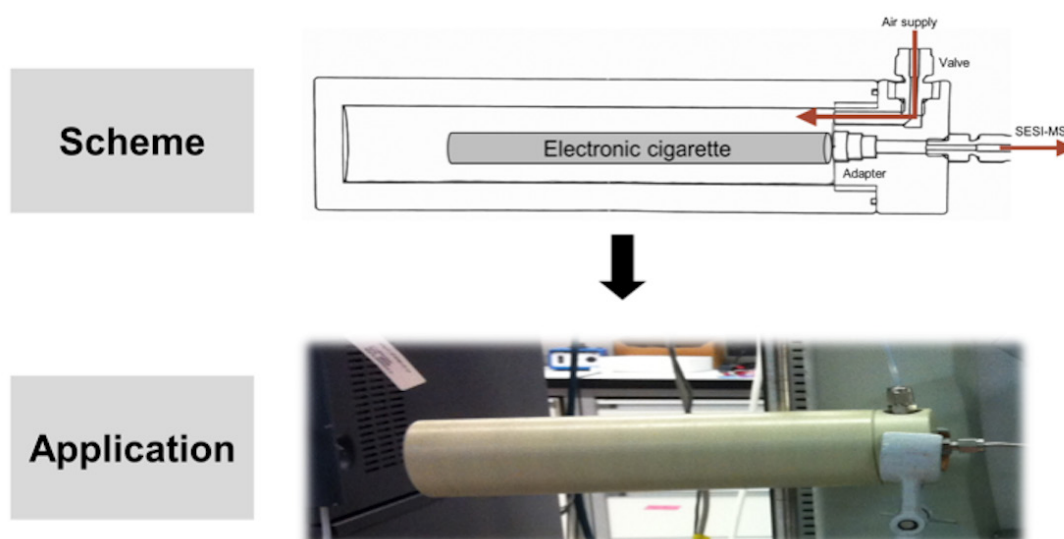


Figure 4. Real-time smoking device developed for realistically simulating the smoking process of electronic cigarettes. After air inlet (synthetic air) the vapor was analyzed in real time by secondary electrospray ionization mass spectrometry.

The SESI smoking device developed was coupled to a LTQ Orbitrap mass spectrometer (Thermo Fisher Scientific, MA, USA) working at a resolution of 100,000 at m/z 400. Spectra from 50 u to 500 u were recorded with a scan time of 200 ms. The peak at 149.0233 (phthalic anhydride) was used as internal lock mass, resulting in a mass accuracy below 2 ppm. Other SESI parameters were: a voltage of 5 kV, a sweep gas flow rate of 2 arbitrary units of nitrogen, and a capillary voltage and temperature of 20 V and 225°C, respectively.

A similar smoking device (“Fast-flow tube”) has recently been proposed by Blair et al.^[29] to allow cigarettes to be analyzed via proton-transfer-reaction mass spectrometry (PTR-MS), another technique for the analysis of vapors in real time. In comparison with SESI, PTR has a similar sensitivity and allows a more direct quantification but it is only capable of analyzing compounds up to 150-200 u, which limits the determination of chemicals such as nicotine. It also presents a much lower power to identify unknown compounds because of its lower mass accuracy and resolution.

Chemicals

Standards of nicotine and myosmine were purchased from Sigma-Aldrich (St. Louis, MO, USA). Tetramethylpyrazine, acetylpyrazine, ethylmaltol, isoamyl isovalerate and ethyl isovalerate standards were purchased from TCI (Eschborn, Germany). Calibration standards were prepared by diluting or dissolving the appropriate amount of standard in water and were used as EC-liquids without further treatment.

Analysis of EC aerosols

For analysis of EC aerosols, the EC reservoir, previously washed with methanol and dried with nitrogen to avoid carry-over, was filled with the appropriate EC-liquid, diluted 1:1000 with water to avoid signal saturation. Since this dilution factor may result in changes in the aerosol composition, some samples were also run undiluted. These runs showed no significant differences in their spectra when compared with the corresponding diluted samples. However, the instrument signal got saturated and a waiting time of 60 minutes between puffs had to be set to avoid carry-over. Therefore, further samples were run with diluted liquids. This 1000-fold dilution should also correct for any matrix effects that may have impaired quantification. E-cig heaters were manually activated immediately before closing the smoking device. Puff duration was set to 5 seconds (controlled by the auto shutdown of the e-cig) and a waiting time of ca. 1 minute was established between puffs. All measurements were repeated at least three times. For targeted quantification, the signals of standard solutions of known concentration were used to build linear calibration curves. The software MZmine 2 (<http://mzmine.github.io>) was used for data analysis (deconvolution and peak list visualization).

Analysis of EC-liquids by ESI-HRMS

For comparison purposes, diluted EC-liquids (1:1000 in water) were also analyzed by means of ESI-HRMS. Samples were directly injected by a Hamilton syringe (5 $\mu\text{L min}^{-1}$) to a standard ESI source (Ion Max, Thermo) coupled to a LTQ Orbitrap mass spectrometer. ESI and Orbitrap parameters were the same as described before for the SESI analysis in order to minimize any influence from solvents and voltages and ensuring, in this way, a proper comparison.

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Supporting Information available: Full experimental details, lists of putatively identified and unidentified chemicals substances found in e-cigs and regression between intensities and output power.

Keywords: Analytical methods • Mass spectrometry • Electronic cigarettes • Nicotine • Secondary electrospray

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