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Evaluation of flavour profiles in e-cigarette refill solutions using gas chromatography–tandem mass spectrometry

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ABSTRACT

Many flavour compounds that are present in e-liquids for e-cigarettes are responsible for specific tastes and smoking sensations for users. Data concerning content and specific types of flavours is often limited and unknown to users. The aim of the research was to define and compare flavour profiles of e-liquids with the same group taste from different manufacturers. Gas chromatography coupled with tandem mass spectrometry (GC–MS/MS) was used to separate and identify 90 popular compounds (98, including isomers) of interest. The developed method was validated in terms of accuracy (88–113%) for three spiking levels and the intra-day (0.2–13%) and inter-day precision (1–10%). Limits of quantitation were in the range of 10–816 ng/mL, while the matrix effects for 80% of the compounds were at negligible levels. The proposed method is rapid, simple and reliable and uses a green and modern GC–MS/MS technique. Twenty-five samples of five different flavours (tobacco, strawberry, cherry, menthol and apple) from five different producers were analysed, and the determined compounds were categorized and differentiated. The approach proposed in this study allowed for the evaluation of which compounds/group of compounds are responsible for taste and to distinguish common flavour compounds among the investigated brands for each flavour. Furthermore, the presented research can be considered in future toxicological studies.

1. Introduction

The availability and growing popularity of flavoured refill liquids for e-cigarettes, especially among young people, raises questions about potential adverse health implications to primary users and non-smokers exposed to second-hand smoke [1,2]. According to the Centers for Disease Control and Prevention (CDC), the use of e-cigarettes (used on at least 1 day during the past 30 days) increased dramatically from 1.5% to 11.3% among US middle and high school students during the period of 2011–2016 [3]. In turn, Filippidis et al. reported that the use of e-cigarettes in 27 European Union (EU) member states increased from 7.2% to 11.6% between 2011 and 2014 [4]. Notably, 81.5% of young e-cigarette users declared they use e-cigarettes "because they come in flavours I like" [5]. Manufacturers offer a wide range of flavoured refill liquids, attracting youths to e-smoking and possibly facilitating the transition to conventional smoking [6,7].

The EU Tobacco Product Directive (2014/40/EU) prohibits implementation of flavoured cigarettes; however, this regulation does not consider e-cigarettes. Moreover, there were over 7700 unique flavoured e-liquids being sold on the market according to a study conducted in 2014 [8]. In view of the above information, a chemical analysis is necessary to determine the flavour components added to e-liquids. Moreover, there is lack of studies on the effects of these additives on human cells, particularly in the lungs [9,10]. The dependence between the possible toxicity and flavour compound concentration in e-liquids is another face that requires attention [2]. Currently, there is limited data on the compounds that are responsible for specific e-liquid flavours. However, a qualitative method for the description of flavours in tobacco products has been presented [11] together with quantitative methods for the determination of the compounds in e-liquids [12–14].

The application of gas chromatography coupled to mass spectrometry (GC–MS) has aroused significant interest in the analysis of flavouring chemicals in various matrices [15–18], including e-liquids [12,14,19–21]. In the last decade, gas chromatography–tandem mass spectrometry (GC–MS/MS) has emerged as a beneficial tool in the analysis of environmental, food and forensic samples [22–24]. The use of MS/MS allows for better selectivity and sensitivity than MS-based methods, and in most cases, reduces interferences, which is crucial in multivariate flavour determination.

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The aim of the present study was to evaluate the taste profiles of e-liquids and to distinguish for the first time the dependence between the e-liquid composition and a given flavour. Although, the number of flavour chemicals is large, more knowledge about the specific and known flavour profiles for a specific flavour will help to introduce additional regulation to ensure the chemicals added to e-liquids are maintained at non-toxic doses.

For this purpose, a new, highly sensitive and robust GC–MS/MS-based method was developed, which allows for the quantification of 90 (identification of 98, including isomers) flavouring chemicals in e-liquids during a single analytical run. The analytes were chosen among the compounds detected in previous studies and from commonly used flavour additives to e-liquids [11–14,19]. To the best of the authors' knowledge, there are no scientific reports regarding the quantitation of a wide range of flavour compounds with the aid of GC–MS/MS. The applicability of the developed method was demonstrated through the analysis of 25 e-liquid samples with an expected characteristic flavour profile (menthol, apple, tobacco, strawberry and cherry) of 5 different brands.

2. Materials and methods

2.1. Reagents and standards

All standards were of analytical grade and obtained from Sigma Aldrich (St. Louis, USA): 1-amyl alcohol, 2-isopropyl-5-methyl-2-hexenal, 3,4-dihydrocoumarin, 2-acetylpyrazine, 2-acetylpyridine, 2-acetylpyrrole, 2-isopropyl-4-methylthiazole, 2-methylpyrazine, 2,5-dimethylpyrazine, 2,6-dimethylpyridine, ethyl 2-methylpropanoate, 2,3,5-trimethylpyrazine, 2,3,5,6-tetramethylpyrazine, 3-ethylpyridine, ethyl-3-methyl-3-phenylglycidate, 4-methyl acetophenone, 5-methylfurfural, anisyl acetate, benzaldehyde, α,α-dimethylphenethyl butyrate, benzyl acetate, benzyl alcohol, capric acid, carvone, citral (mixture of *cis and trans;* neral and geranial), citronellol,-3 *cis*-3-hexenyl acetate, *cis*-3-hexenyl-valerate, cocal, decanal, diethyl malonate, diethyl succinate, ethyl caproate, ethyl cinnamate, ethyl lactate, ethyl heptanoate, ethyl phenylacetate, ethyl vanillin, ethyl 3-(methylthio)propionate, ethyl maltol, eugenol, furaneol, furfural, furfuryl alcohol, geranyl propionate, geraniol, hedione (mixture of *cis* and *trans*), hexyl acetate, hexyl hexanoate, ionone α, ionone β, isoamyl butyrate, isoamyl isovalerate, isopentyl acetate, leaf aldehyde, leaf alcohol (*cis*-3-hexen-1-ol), limonene, linalool, linalool oxide, linalyl acetate, L-menthyl acetate, maltol, melonal, menthol, menthone, methyl cinnamate, methyl cyclopentenolone, methyl heptenone, methyl salicylate, nerol, *n*-hexanol, phenethyl alcohol, phenethyl isovalerate, raspberry ketone, styralyl acetate, tetrahydrolinalool, theaspirane,-2 *trans*-2-hexenol, vanillin, β-damascone, δ-tetradecalactone, γ-valeroactone, γ-hexalactone, α-terpineol, γ-nonanolactone, γ-butyrolactone, γ-decalactone, γ-dodecalactone and δ-decalactone. Naphthalene-d_8 was used as the internal standard (IS) and was purchased from Isotec/Sigma-Aldrich (St. Louis, USA). Vegetable glycerine (VG) and propylene glycol (PG) were purchased from Anwit (Warsaw, Poland), and acetonitrile (ACN) (MS grade) was obtained from Merck (Darmstadt, Germany).

2.2. Standard solutions, calibration solutions and validation formulations

Standard stock solutions of the flavours and the IS (naphthalene-d_8) were prepared separately in ACN (5 mg/mL). The working standard mixture of the analytes was prepared by mixing and diluting the standard stock solutions with ACN to obtain a concentration of 25 μg/mL of each substance.

According to the labels, the main component of samples of e-liquids are: propylene glycol (above 50%), glycerine (content described as low 1–30% or in most cases as medium 30–50%) and water (content described as below 10%). The samples used for method validation were prepared as follows: 100 mg of blank laboratory made e-liquid (65% propylene glycol, 30% glycerine, 5% water, w/w/w) was spiked with an aliquot of the working standard mixture to obtain three spiking levels: 0.01, 0.08, 0.4 mg/mL, what corresponds to 100, 800 and 4000 ng/mL in the samples with a 100 x dilution factor.

For furfuryl alcohol and capric acid, different spiking levels were applied: 0.03, 0.08 and 0.4 mg/mL and 0.08, 0.15 and 0.4 mg/mL, respectively. Seven calibration solutions (n = 3) were prepared using a laboratory made e-liquid as the matrix in concentration ranges specific for each compound (in general, 50–5000 ng/mL). The concentration of the IS in every solution was maintained at 500 ng/mL. Validation samples were used for the evaluation of the accuracy, precision and matrix effects of the developed procedure.

2.3. E-liquid samples

All commercially available e-liquids samples were purchased from five companies present on the Polish market. The selection of e-liquids was based on the brand and flavour popularity. The selection of flavour was performed based on questionnaires carried out among sellers from local stores in Gdańsk about which flavours and brands are the most frequently bought by users of e-cigarettes. Five e-liquids, which were expected to have a characteristic flavour (menthol, apple, tobacco, strawberry and cherry), were purchased from each manufacturer. For every e-liquid, the information pertaining to the presence of the primary components was provided (glycerine, propylene glycol and nicotine). In most cases, the composition (without concentration) of the flavouring compounds was included. A total of 25 e-liquid samples were selected, and the samples were analysed within two weeks of purchase after storage at room temperature in a dark place similar to the shop conditions.

2.4. Sample preparation

E-liquid samples were prepared prior to the analysis according to a recently published procedure [12]. Briefly, approximately 100 mg of an e-liquid sample was weighed into a 10-mL volumetric flask. Subsequently, the IS was added, and the flask filled to the mark with ACN.

2.5. GC–MS/MS conditions

The GC–MS/MS analyses were performed on a Shimadzu GC-2010 PLUS System (Kyoto, Japan) coupled with a Shimadzu TQ8050 triple quadrupole mass spectrometer (Kyoto, Japan). The separation of the analytes was performed on a Phenomenex ZB-5 MSi (30 m × 0.25 mm i.d., 0.25 μm film thickness) capillary column. Helium (purity ≥99.999%) was applied as a carrier gas at a constant flow rate of 1 mL/min. The temperature programme was set as follows: 50 °C (hold for 4 min), ramp to 130 °C at 10 °C/min, then to 300 °C (hold for 3 min) at 25 °C/min. The injection volume was 1 μL in the splitless injection mode (1 min), and a solvent delay time of 2.6 min was used. The temperature of the transfer line and injector temperature was set at 285 °C and 250 °C, respectively. The MS was operated in electron impact (EI) mode with an electron energy of 70 eV, and the ion source temperature was set at 220 °C. Argon (purity ≥99.999%) was applied as the collision-induced dissociation (CID) gas with a scan range that covered 30–250 *m/z*. For quantitation and validation, the instrument was operated in multiple reaction monitoring mode (MRM). The monitored transitions and optimized collision energies (CEs) are listed in Table 1. For