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Comprehensive determination of flavouring additives and nicotine in e-cigarette refill solutions. Part I: Liquid chromatography-tandem mass spectrometry analysis

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a b s t r a c t

Liquid chromatography-tandem mass spectrometry with electrospray ionization (HPLC-ESI–MS/MS) methods were developed for the simultaneous determination of 42 flavouring compounds and nicotine in liquids for e-cigarettes. The chromatographic separation was performed using an Ace® Ultracore™ SuperC18™ (100 × 2.1 mm, 2.5 □ m) column in both acidic and alkaline pH conditions to separate all the compounds. A simple "dilute & shoot" approach was used for the sample preparation. The method validation was performed by evaluating key analytical parameters such as linearity, accuracy, selectiv-ity, precision, limit of detection (LOD) and limit of quantification (LOQ). The calibration curves showed good linearity within the specific ranges for the investigated compounds with correlation coefficients greater than 0.990 in each case. The recovery for all the investigated compounds varied from 89% to 110%. The intra- and inter-day precision were within the acceptable limits (±15%) at all tested concen-trations. The applicability of the methods was examined by analysing 25 liquid samples from e-cigarettes commercially available on the Polish market.

Keywords: Electronic cigarettes Liquid, chromatography Tandem, mass spectrometry, Flavouring compounds, Nicotine

1. Introduction

Tobacco smoking is one of the most serious public health threats that the world has ever faced. Smoking causes more than 6 million deaths each year all over the world, and more than 600,000 deaths are the result of non-smokers being exposed to secondhand smoke [1]. This clearly shows that despite society's growing awareness of the consequences of excessive smoking, the problem remains unsolved. In the last several years, electronic nicotine delivery systems (ENDS), also referred to as electronic cigarettes, have become popular with millions of users all over the world. ENDS are marketed as a healthier alternative to conventional smoking due to the lack of tobacco combustion, i.e., the formation of carcinogenic compounds is limited [2–6]. The promotion in the media of e-cigarettes as a harmless substitute for traditional smoking has led to some controversies about their unknown long-term safety. The rapid expansion of the e-cigarette market and the increasing number of various replacement liquids require that new analytical methods be developed to control the quality of such products [7–9].

The liquid used in e-cigarettes is usually a mixture of propylene glycol and/or vegetable glycerine, ethanol, water, nicotine and flavours (for example: tobacco, vanilla, coffee, chocolate) [10,11]. The number of flavoured liquids present on the market is staggering – approximately 7700 are currently on sale [12]. Flavour compounds used in e-cigarettes are considered as food grade or generally recognized as safe (GRAS) by FEMA (Flavour and Extract Manufacturers Association). However, the safety of these flavours refers to human exposure through ingestion, not inhalation [13,14]. Currently, relatively little is known about the possible impact of flavours transformed from an e-liquid to an aerosol that is then taken up by cells in the lungs [15]. Moreover, these compounds may interact with other components of the e-liquid during vaporization and could be converted into toxic products with different physiochemical properties [13]. The concentrations of the flavour compounds are often not given on the labels of replacement liquids, and some of the compounds might have toxic or irritating potencies [16–19]. These findings may prove that there is a potential threat to human health and toxic effects from the flavouring compounds inhaled during the use of e-cigarettes. To fill in the information gap about the safety of e-cigarettes and to influence future regulations, there is a need for research to characterise the full profile of flavouring chemicals used in e-liquids [20,16].

Based on the literature data, only a few methods for the determination of flavour chemicals in e-cigarette products have been published. To the best of the authors' knowledge, there is only one method utilizing a LC–MS technique for the determination of

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flavourings in e-liquids [21]. However, the mentioned study was limited to a small target list of 5 main flavour ingredients: ethyl maltol, ethyl vanillin, 2,5-dimethylpyrazine, methyl cyclopentenolone and 3,4-dimethoxybenzaldehyde. In view of the above, there is an urgent need to extend the list of the analytes of interest that may have potential effects on human health. In this study, a LC–MS/MS technique was applied because it provides improved reproducibility, specificity and sensitivity compared to LC–MS. These parameters are crucial in an efficient implementation of a simultaneous multicomponent analysis.

The purpose of this project was to develop and validate a rapid, selective and sensitive LC–MS/MS method in a multiple reaction monitoring (MRM) mode for simultaneous determination of 42 flavouring compounds and nicotine in replacement liquids.

2. Material and methods

2.1. Chemicals

All standards investigated in the study were obtained from Sigma-Aldrich (St. Louis, USA): 2-acetylpyrazine, 2-acetylpyridine, 2-acetylpyrrole, 2-isopropyl-4-methylthiazole, 2-methylpyrazine, 2,5-dimethylpyrazine, 2,6-dimethylpyridine, 2,3,5-trimethylpyrazine, 2,3,5,6-tetramethylpyrazine, 3-ethylpyridine, 3-methyl-3-phenylglycidate, 4-methyl acetophenone, 5-methylfurfural, carvone, cocoa, diethyl malonate, diethyl succinate, ethyl acetoacetate, ethyl cinnamate, ethyl lactate, ethyl phenylacetate, ethyl vanillin, ethyl-2-methylbutyrate, ethyl 3-(methylthio)propionate, ethyl maltol, furaneol, geraniol, ionone α, ionone β, linalool, linalool oxide, maltol, menthol, menthone, methyl cinnamate, methyl cyclopentenolone, methyl heptenone, methyl salicylate, nerol, nicotine, pyridine, vanillin, β-damascone, γ-valeroactone, and γ-hexalactone. HPLC-grade formic (FA) and acetic acid (AA) and acetonitrile HPLC gradient (ACN) were obtained from Merck (Darmstadt, Germany). Ammonium bicarbonate, ammonium formate and ammonium acetate (analytical grade) were obtained from Sigma Aldrich (St. Louis, USA). Vegetable glycerine and propylene glycol were purchased from Anwit (Warsaw, Poland). Ultrapure water was produced in an HLP_5 system Hydrolab (Straszyn, Poland).

2.2. Samples

Twenty-five e-liquids were selected as representatives of different flavour groups. The choice of e-liquids was dictated by labels covering the following flavours: fruit, tobacco, menthol and special. No or limited information was provided on the bottle containing the e-liquid or the label about the flavours attached to the product. However, all the products were labelled with information about the nicotine content.

2.3. Standards and calibration solutions

Individual stock solutions of the flavouring compound standards, nicotine and internal standard (IS) were prepared by diluting or dissolving the standards in ACN at a concentration of approximately 5 mg/mL. Cyclophosphamide was used as the internal standard (IS). Working standard mixtures of the analytes were prepared by diluting the stock solutions in ACN to obtain a final concentration of 50 μg/mL for each analyte. The calibration solutions were prepared by diluting the working standard solution in a mixture of ACN and water (1:9, v/v) to prepare calibration solutions in a specific calibration range for each compound. A stock solution of the internal standard was prepared at a concentration of 100 μg/mL and used in all the analyses. In each calibration solution, the concentration of IS was maintained at 200 ng/mL. All the stock solutions and working solutions were stored in a refrigerator at +4 °C until they were used.

2.4. Sample preparation and fortified samples

An appropriate amount of the e-liquid samples was weighed in a 50 mL volumetric flask and diluted with a mixture of ACN:H_2O (1:9, v/v) to ensure the concentration fell within the calibration curve range specified for each compound. A 100-fold dilution was appropriate in most cases. To evaluate the concentration of nicotine, a 10,000-fold dilution was applied to avoid saturation of the detector. Subsequently, the internal standard (IS) solution was added to obtain a concentration of 200 ng/mL, and the flask was filled with a mixture of ACN:H_2O (1:9, v/v).

To estimate the accuracy and precision of the developed method, a homemade e-liquid was created and used as an analytical control. The preliminary studies showed that the investigated e-liquids were mainly composed of propylene glycol (≤65%) and vegetable glycerine (≤30%) and traces of nicotine and flavours. Therefore, the homemade e-liquid contained 65% propylene glycol, 30% vegetable glycerine and 5% water and was used as a blank matrix and for the fortification procedures.

The fortified samples were prepared by adding appropriate amounts of the standards into the laboratory-made e-liquid to obtain three different concentration levels (low, medium and high) within the specific linear range for each compound. Fortified samples were prepared as mentioned above and were used to evaluate the repeatability and accuracy of the method.

2.5. MS/MS parameters

All analyses were performed using a LCMS-8060 triple quadrupole mass spectrometer (Shimadzu, Japan) equipped with an ESI source working in a positive MRM ion mode. The parameters of the ion source were set as follows: nebulizing gas flow: 3 L/min; heating gas flow: 10 L/min; drying gas flow: 10 L/min; interface temperature: 300 °C; heat block temperature: 400 °C and (desolvation line) DL temperature: 250 °C. Data acquisition and quantification were accomplished using LabSolutions 5.85 software. The specific MRM conditions and ion source parameters were specified via an infusion of a 1 μg/mL solution of each substance by flow injection analysis (FIA). For most of the compounds, the acquisition was performed in MRM mode, and two transitions were monitored. The transition with the highest intensity was used for quantification, and the second most intense transition was used for confirmation. To increase the sensitivity and achieve a better S/N (signal to noise ratio), the MRM transitions were monitored only for specific detection time frames around the analytes' expected retention times. Additionally, to verify the presence of the flavour compounds in each sample, the quantifier/qualifier ion ratio was monitored. Positive identification was performed if the established MRM ratios in the investigated e-liquid samples were within ± 20% of those used after the standard solution analysis. However, a few analytes only exhibited fragmentation to a small extent, and for those compounds, a single MRM transition was monitored. The optimum detection conditions are presented in Table S1 (Electronic Supplementary Material).

2.6. HPLC conditions

Chromatographic separation of the analytes was performed on an UPLC system (Shimadzu, Japan) equipped with a degasser DGU-20A5R, controller CBM-20A, two pumps Nexera X2 LC-30 CE, autosampler Nexera X2 SIL-30AC and column oven CTO 20AC. Two different chromatographic methods utilizing the different pH values of the mobile phase were developed using the same column to ensure separation and reliable quantification of the investigated analytes. The separation in both cases was achieved using an Ace® Ultracore™ SuperC18™ column (100 × 2.1 mm, 2.5 μm in core-

Table 1
Chromatographic separation conditions.

	Acidic conditions	Alkaline conditions
Chromatographic column	Ace® Ultracore™ SuperC18™ (100 × 2.1 mm, 2.5 μm)	
Mobile phase composition	A: H_2O 0.05% v/v of FA (pH = 3) B: ACN 0.05% v/v of FA	A: 10 mM NH_4HCO_3 (pH = 10.5) B: ACN
Flow	0.8 mL/min	
Column oven temperature	30 °C	
Injection volume	5 μL	
Mobile phase gradient	0–2 min (5% B) 2–12 min (5–90%B)	0–2 min (12% B) 2–5 min (12–42% B)
Equlibration time	5 min (5% B)	

shell technology) equipped with a precolumn and maintained at 30 °C. The chromatographic separation conditions for each chromatographic method are presented in Table 1.

2.7. Method validation procedures

The method described in this study was validated in terms of selectivity, linearity, LODs, LOQs, accuracy and precision according to the guidelines for analytical method validation [22–24]. Selectivity experiments were performed to evaluate the presence of interfering peaks in the retention times of the investigated analytes and IS. For this purpose, six blank matrix samples containing neither the analyte or IS were analysed.

Six-point calibration curves were constructed (n = 3) based on the analysis of the analyte solutions by plotting the ratio of the analyte peak area to the IS peak area versus the concentration using a linear least squares regression with a proper weighting factor. The linearity of the calibration curves was investigated in the concentration range specific for each compound. Cyclophosphamide was chosen as the IS due to its absence in e-liquid samples and for its compensation of the detector response.

The LOD values for each analyte were calculated based on the formula: $LOD = 3.3^{*}\ S_b/a$, where a is the slope of the calibration curve, and S_b is the standard deviation of the intercept. The LOQ values were estimated by multiplying the LOD values by 3.

The accuracy of the method was assessed by replicate analyses (n = 3) of the fortified e-liquid at three concentration levels within the specific linear range for each compound. The accuracy was calculated as the percent (%) difference between the measured concentration and nominal concentration. The intra-day precision was evaluated by analysing three replicates at the previously mentioned concentration levels during one day and was expressed as the coefficient of variation (CV). Inter-day precision of the method was performed by analysing a batch of samples (n = 6) at a medium concentration level for three consecutive days.

3. Results and discussion

3.1. Separation conditions

Developing a method for a large number of compounds with varying polarities and solubilities is a challenging task. One of the main objectives of this research was to separate all the investigated compounds. The method was developed using an Ace® Ultracore™ SuperC18™ column with core shell-technology that works over a wide pH range (1.5-11.5). To achieve separation and appropriate peak shapes, several operational parameters, including different mobile phase compositions (acetic and formic acid additions, ammonium formate and acetate buffers, ammonium bicarbonate buffer, MeOH, ACN), gradients, and temperatures, were extensively tested. Due to the different polarities of the tested compounds, gradient elution was used to achieve short analysis times of less than 10 min. Many attempts were made to choose an appropriate organic phase as the eluent. In particular, MeOH, ACN and mixtures of the two at different proportions were examined as the organic component of the mobile phase. The use of ACN as the organic component of the mobile phase resulted in better peak shapes and resolution compared with methanol. Also, the addition of AA and FA to the mobile phase in the range from 0.01 to 0.1% was examined. Satisfying results in terms of the separation and signal response for all analytes detected in the ESI+ mode were observed after the addition of formic acid at 0.05% (v/v) to water and ACN. Ammonium acetate or ammonium formate buffer (10, 25, 50 mM) were tested as the aqueous components of the mobile phase, but they did not affect the sensitivity or the peak shapes in comparison to the results obtained with the addition of FA. The optimum temperature was 30 °C, and increasing the temperature did not positively affect the peak shapes. Following the subsequent optimization of the gradient elution programmes, the final mobile phase consisted of ACN and H_2O with 0.05% v/v of FA.

Two pairs of compounds, α- and β- ionone and nerol and geraniol, are isobaric geometric (*cis, trans*) isomers of each other. Therefore, both exhibit the same CID-fragmentation pattern, and they cannot be separated using the LC technique and conditions utilized in this study. Hence, in these two cases, the results were expressed as the sum of the unresolved isomers.

However, regardless of the acidic conditions tested, the separation of 2,5-dimethylpyrazine (pKa = 1.59), 2,6-dimethylpyridine (pKa = 6.54), nicotine (pKa = 8.86), pyridine (pKa = 5.12) and 3-ethylpyridine (pKa = 5.57) was not achieved, and these analytes eluted with the void volume of the column (Fig. 1A). Under the tested acidic conditions (pH = 3), these compounds exhibited minimal retention because they are mostly in their ionized forms. To obtain the desired separation, a second, complementary chromatographic method utilizing a mobile phase with a high pH was developed. The mobile phase consisted of 10 mM ammonium bicarbonate at pH = 10.5 (A) and ACN (B). As the mobile phase increased from pH 3.0–10.5, the analytes became more hydrophobic (non-ionized) and their retention increased. The adjustment in the selectivity was possible with the use of the Ace® Ultracore™ SuperC18™ column, which is designed to be stable under acidic and high pH conditions with LC–MS compatible buffers. This enhanced stability is ensured by the encapsulated bonding technology, which significantly minimizes the negative effects of unbound silane groups. The high ligand coverage results in improved inertness, efficiency, peak shape and reproducibility. The final chromatographic conditions for the two methods are summarized in Table 1. Chromatograms of the standard mixtures obtained under acidic and alkaline conditions are presented in Fig. 1B and C.

3.2. Assay validation

3.2.1. Selectivity, linearity, LOD, LOQ

The developed methods were selective for the investigated compounds, and no interfering peaks were observed in the MRM chromatograms at the expected retention times for the analytes.

The calibration curves were created by plotting the ratio of the analyte peak area to the IS peak area against the analyte concentration over the specific concentration range for each analyte, as listed

Table 2
Quantification parameters for investigated flavouring compounds and nicotine.

Lp.	Analyte	CAS number	Concentration range [ng/mL]	Calibration curve equations (6 points, n = 3)	Sa	Sb	r	LOD [ng/mL]	LOQ [ng/mL]
1	2-methylpyrazine	109–08-0	10 – 1000	y = 0.000168x + 0.0009	0.000002	0.0002	0.9949	5	14
2	γ- valeroactone	108–29-2	400 – 2000	y = 0.000060 x – 0.0009	0.000003	0.0027	0.9901	148	444
3	Furaneol	3658–77-3	10 – 1000	y = 0.0002745x + 0.0139	0.000003	0.0004	0.9975	5	15
4	Maltol	118–71-8	5 – 1000	y = 0.00255x + 0.002	0.00001	0.001	0.9977	1	4
5	2-acetylpyrazine	22047–25-2	5 – 1000	y = 0.001374x + 0.0013	0.000005	0.0005	0.9997	1	4
6	Methyl cyclopentenolone	80–71-7	25 – 2000	y = 0.0000888x + 0.0001	0.0000007	0.0002	0.9948	7	21
7	Ethyl lactate	97–64-3	100 – 2000	y = 0.0000088x + 0.0001	0.0000003	0.0001	0.9919	45	135
8	Ethyl acetoacetate	141–97-9	50 – 2000	y = 0.000161x + 0.0004	0.000003	0.0011	0.9905	21	69
9	2,3,5,6-tetramethylpyrazine	1124–11-4	5 – 1000	y = 0.0205 x – 0.003	0.0001	0.011	0.9993	2	5
10	2-acetylpyridine	1122–62-9	5 – 1000	y = 0.00541 x – 0.002	0.00002	0.002	0.9939	1	4
11	2,3,5-trimethylpyrazine	14667–55-1	5 – 800	y = 0.00263 x – 0.0009	0.00001	0.0010	0.9990	1	4
12	5-methylfurfural	620–02-0	10 – 2000	y = 0.00086x + 0.003	0.00001	0.002	0.9937	6	19
13	γ-hexalactone	695–06-7	100 – 1000	y = 0.000068 x – 0.0006	0.000001	0.0005	0.9898	26	78
14	2-acetylpyrrole	1072–83-9	5 – 1000	y = 0.00328 x – 0.0001	0.00009	0.0013	0.9951	1	4
15	Ethyl maltol	4940–11-8	5 – 1000	y = 0.0161 x – 0.05	0.0003	0.02	0.9960	5	15
16	Vanilin	121–33-5	5 – 2000	y = 0.0011854x + 0.00074	0.0000082	0.00096	0.9959	3	8
17	2-isopropyl-4-methylthiazole	15679–19-3	5 – 800	y = 0.000572x + 0.0009	0.000004	0.0003	0.9977	2	5
18	Ethyl vanilin	121–32-4	5 – 2000	y = 0.00087x + 0.001	0.00001	0.001	0.9944	4	13
19	Diethyl malonate	105–53-3	25 – 2000	y = 0.00394 x – 0.002	0.00004	0.003	0.9896	4	13
20	Diethyl succinate	123–25-1	5 – 800	y = 0.00363x + 0.003	0.00007	0.002	0.9976	3	8
21	Methyl salicylate	119–36-8	5 – 800	y = 0.000072x + 0.00045	0.000001	0.00007	0.9968	3	10
22	Linalool oxide	1365–19-1	50 – 1000	y = 0.0000477x + 0.0031	0.0000008	0.0002	0.9892	13	38
23	Ethyl 3-(methylthio)propionate	13327–56-5	5 – 1000	y = 0.0000504 x – 0.00002	0.0000003	0.00003	0.9932	2	6
24	4-methyl acetophenone	122–00-9	10 – 800	y = 0.0000932x + 0.00036	0.0000008	0.00009	0.9974	3	10
25	Methyl heptenone	110–93-0	10 – 1000	y = 0.00102x + 0.035	0.00001	0.002	0.9987	8	23
26	Ethyl-2-methylbutyrate	7452–79-1	25 – 2000	y = 0.0000564x + 0.00004	0.0000005	0.00012	0.9949	7	21
27	Carvone	140–11-4	50 – 2000	y = 0.000130x + 0.003	0.000004	0.001	0.9871	32	96
28	Ethyl phenylacetate	101–97-3	100 – 2000	y = 0.0000151x + 0.001	0.0000002	0.0001	0.9897	24	72
29	Methyl cinammate	103–26-4	50 – 2000	y = 0.0000246x + 0.0015	0.0000004	0.0002	0.9850	21	62
30	Linalol	78–70-6	5 – 2000	y = 0.000731x + 0.0074	0.000004	0.0005	0.9957	2	7
31	Nerol + geraniol	106–25-2	5 – 2000	y = 0.000336x + 0.0023	0.000002	0.0002	0.9940	2	6
32	3-methyl-3-phenylglycidate	93–18-5	5 – 1000	y = 0.0000662x + 0.00064	0.0000006	0.00006	0.9922	3	9
33	Menthol	1490–04-6	200 – 2000	y = 0.0000145 x – 0.0005	0.0000004	0.0003	0.99311	57	170
34	Ethyl cinnamate	103–36-6	25 – 1000	y = 0.0000870x + 0.0004	0.0000006	0.0001	0.9976	5	14
35	Menthone	89–80-5	100 – 2000	y = 0.0000140 x – 0.00038	0.0000001	0.00009	0.9921	54	161
36	Cocal	21834–92-4	25 – 1000	y = 0.0000501x + 0.00034	0.0000005	0.00009	0.9933	6	17
37	Ionone (α + β)	127–41-3	5 – 2000	y = 0.000943x + 0.0187	0.000005	0.0006	0.9944	2	7
38	β –Damascone	23726–92-3	10 – 2000	y = 0.00225x + 0.0003	0.00001	0.0024	0.9986	4	10
39	2,5 –dimethylpyrazine	123–32-0	25 – 2000	y = 0.0000335 x – 0.00021	0.0000003	0.00008	0.9963	8	24
40	pyridne	110–86-1	25 – 2000	y = 0.000478 x – 0.0007	0.000005	0.0004	0.9960	2	7
41	2,6 – dimethylpyridine	108–48-5	5 – 2000	y = 0.00100 x – 0.0032	0.00006	0.0009	0.9957	3	9
42	nicotine	54–11-5	25 – 2000	y = 0.0222 x – 0.06	0.0001	0.02	0.9995	3	8
43	3-ethylpyridine	536–78-7	25–2000	y = 0.000743 x – 0.012	0.000006	0.002	0.9966	7	22

S_a – standard deviation of the slope, S_b – standard deviation of the intercept. *r* - correlation coefficient, *LOD* - limit of detection. *LOQ* – limit of quantitation, *n* - number of measurements.

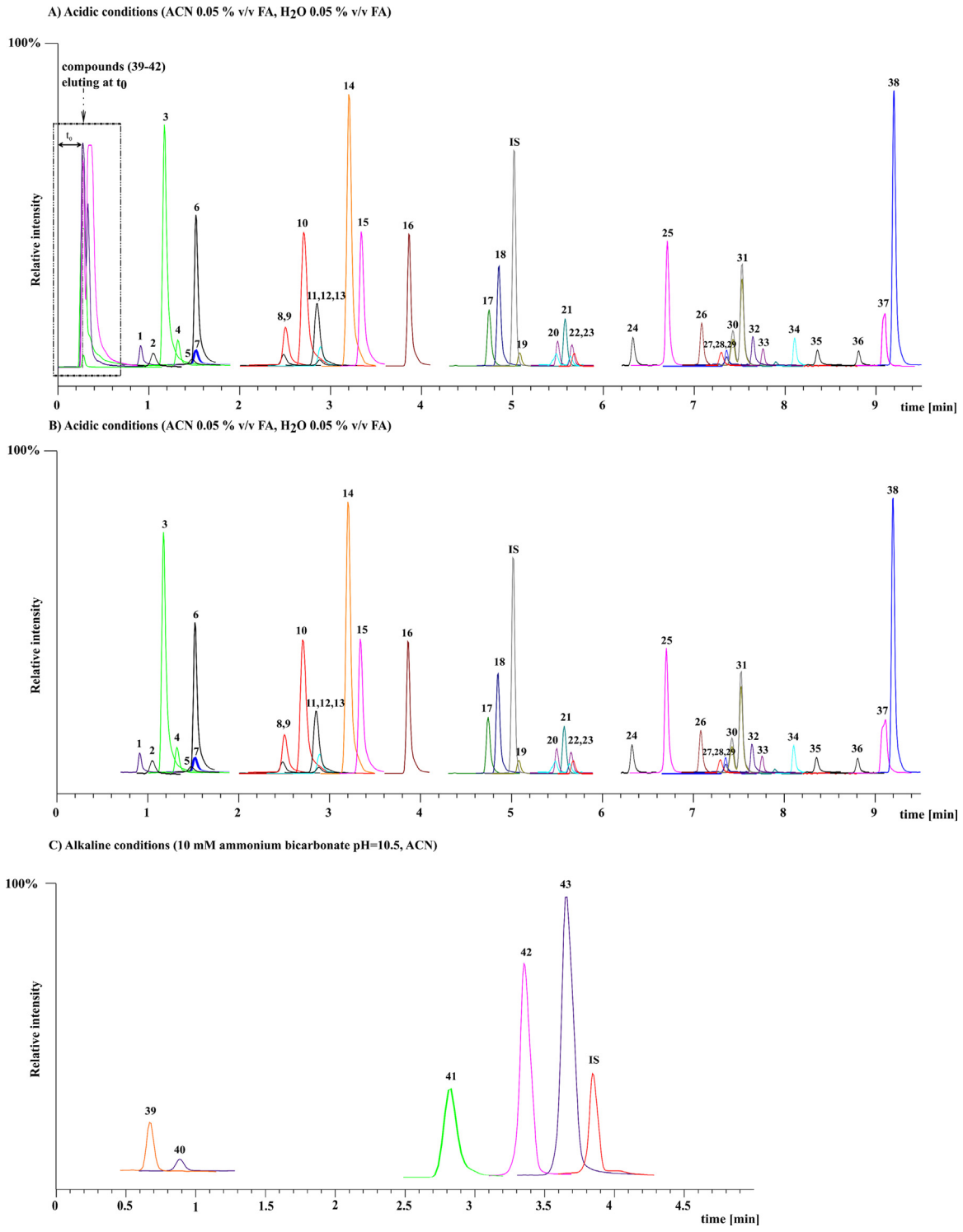

Fig. 1. MRM chromatograms obtained with Ace® Ultracore™ SuperC18™ column (100 × 2.1 mm, 2.5 μm): A) chromatogram of mixture of all analytes at 200 ng/ mL (of each) – acidic conditions, B: final chromatogram of standard mixture at 200 ng/ mL (of each) – acidic conditions, C: mixture of nicotine, 2,6-dimethylpyridine, 3-ethylpyridine, pyridine, 2,5-dimethylpyrazine at 200 ng/ mL (of each) – alkaline conditions.

in Table 2. The calibration curves were linear in the studied concentration ranges, and the correlation coefficients were greater than 0.990 for all the compounds. To improve the accuracy at the lower concentrations in the calibration curve, a weighted linear regression (1/x) was applied. The equations for the calibration curves, LOD and LOQ values, correlation coefficients (r), standard deviations of the slope (S_a) and standard deviations of the intercept (S_b) are summarized in Table 2. The obtained LOD and LOQ values are sufficient to meet the acceptance criteria LOD<C_{min} and 10xLOD>C_{min}. This indicates that the developed methods are sensitive, and it is possible to determine even trace amounts of all the compounds investigated in this study.

3.2.2. *Precision and accuracy*

The intra-, inter-day precision and accuracy results from the two methods are summarized in Table S2 (Supplementary Electronic

Table 3
Concentration of flavouring compounds and nicotine in investigated e-liquid samples. Only detected compounds are listed in Table.

E-liquid sample	Compound detected	Concentration found [mg/mL ± SD (n = 3)]	Concentration found [%w/v ± SD (n = 3)]
Raspberry	Nicotine	17.1 ± 0.3	1.70 ± 0.03
	2-methylpyrazine	0.0434 ± 0.0008	0.00434 ± 0.00008
Devil_1	2,3,5,6-tetramethylpyrazine	0.0420 ± 0.0007	0.00420 ± 0.00007
	2-methylpyrazine	0.0253 ± 0.0008	0.00253 ± 0.00008
	Cocal	0.0197 ± 0.0005	0.00197 ± 0.00005
	Vanilin	0.43 ± 0.01	0.043 ± 0.001
	Ethylvanilin	0.043 ± 0.004	0.0043 ± 0.0004
	2,3,5-trimethylpyrazine	0.043 ± 0.004	0.0043 ± 0.0004
Devil_2[b]	Nicotine	17.3 ± 0.5	1.73 ± 0.05
	2,3,5,6- tetramethylpyrazine	0.051 ± 0.004	0.0051 ± 0.0004
	2-methylpyrazine	0.0267 ± 0.0008	0.00267 ± 0.00008
	Cocal	0.0035 ± 0.0002	0.00035 ± 0.00002
	Vanilin	0.41 ± 0.03	0.041 ± 0.003
	2,3,5-trimethylpyrazine	0.056 ± 0.004	0.0056 ± 0.0004
	Ethyl maltol	0.0011± 0.0001	0.00011 ± 0.00001
Vanilla	Nicotine	18.3 ± 0.3	1.83 ± 0.03
	Ethylvanilin	2.00 ± 0.02	0.200 ± 0.002
	Ethyl maltol	0.19 ± 0.01	0.019 ± 0.001
	Vanilin	2.3 ± 0.1	0.23 ± 0.01
	γ-valeroactone	0.088 ± 0.004	0.0088 ± 0.0004
	Carvone	(0.0085)[a]	(0.00085)[a]
	Menthol	0.132 ± 0.007	0.0132 ± 0.0007
Strawberry	Nicotine	18.5 ± 0.3	1.85 ± 0.03
	Furaneol	0.094 ± 0.003	0.0094 ± 0.0003
	Ethyl lactate	0.05 ± 0.01	0.005 ± 0.001
	Ethyl maltol	0.216 ± 0.007	0.0216 ± 0.0007
	2-methylbutyrate	0.157 ± 0.007	0.0157 ± 0.0007
	Methyl cinnammate	0.187 ± 0.009	0.0187 ± 0.0009
Apple	Nicotine	18.4 ± 0.9	1.84 ± 0.09
	Ethyl acetoacetate	15.1 ± 0.6	1.51 ± 0.06
	Vanilin	0.032 ± 0.002	0.0032 ± 0.0002
	β-damascone	0.0016 ± 0.0001	0.00016 ± 0.00001
	Linalool oxide	0.0060 ± 0.0007	0.00060 ± 0.00007
	Ethyl phenylacetate	0.021 ± 0.003	0.0021 ± 0.0003
	Maltol	0.95 ± 0.04	0.095 ± 0.004
	Furaneol	0.020 ± 0.001	0.0020 ± 0.0001
Black currant	Nicotine	17.8 ± 0.4	1.78 ± 0.04
	Linalol	0.154 ± 0.003	0.0154 ± 0.0003
	Furaneol	0.019 ± 0.001	0.0019 ± 0.0001
	Ethyl maltol	0.093 ± 0.005	0.0093 ± 0.0005
	4-methylacetophenone	0.063 ± 0.003	0.0063 ± 0.0003
	Cocal	0.036 ± 0.003	0.0036 ± 0.0003
	Methylcyclopentenolone	0.0058 ± 0.0007	0.00058 ± 0.00007
Camel	Nicotine	17 ± 2	1.7 ± 0.2
	Ethyl maltol	3.60 ± 0.05	0.360 ± 0.005
	2,3,5-trimethylpyrazine	0.0731 ± 0.0007	0.00731 ± 0.00007
	β-damascone	0.071 ± 0.001	0.0071 ± 0.0001
	Ethylacetoacetate	0.066 ± 0.002	0.0066 ± 0.0002
	Maltol	0.0041 ± 0.0002	0.00041 ± 0.00002
	Vanilin	0.00084 ± 0.00007	0.000084 ± 0.000007
	Linalool oxide	0.037 ± 0.003	0.0037 ± 0.0003
	Methylcyclopentenolone	0.0067 ± 0.0008	0.00067± 0.00008
Elem_1	Nicotine	15.4 ± 0.4	1.54 ± 0.04
	Pyridyne	0.0124 ± 0.0007	0.00124 ± 0.00007
	β-damascone	0.0041 ± 0.0002	0.00041 ± 0.00002
	2,5-dimethylpyrazine	0.013 ± 0.002	0.0013 ± 0.0002
	2-acethylpyrazine	0.039 ± 0.002	0.0039 ± 0.0002
	2,3,5-trimethylpyrazine	0.039 ± 0.002	0.0039 ± 0.0002
	2-acetylopirol	0.021 ± 0.001	0.0021 ± 0.0001
	Methylcyclopentenolone	0.019 ± 0.001	0.0019 ± 0.0001
	Ethyl maltol	0.040 ± 0.001	0.0040 ± 0.0001
	2,6-dimethylpyridine	(0.0012)[a]	(0.00012)[a]
Elem_2[b]	Pyridyne	0.0087 ± 0.0003	0.00087 ± 0.00003
	2,3,5-trimethylpyrazine	0.044 ± 0.002	0.0044 ± 0.0002
	2,5-dimethylpyrazine	0.010 ± 0.002	0.0010 ± 0.0002
	2-acethylpyrazine	0.040 ± 0.002	0.0040 ± 0.0002
	2-acetylopirol	0.0211 ± 0.0004	0.00211 ± 0.00004
	Methylcyclopentenolone	2.9 ± 0.2	0.29 ± 0.02
	β-damascone	0.0043 ± 0.0002	0.00043 ± 0.00002
	Ethyl maltol	0.042 ± 0.003	0.0042 ± 0.0003
Standard_1	Nicotine	17.6 ± 0.6	1.76 ± 0.06
	Methylcyclopentenolone	0.074 ± 0.006	0.0074 ± 0.0006
	Ethyl maltol	1.07 ± 0.05	0.107 ± 0.005
	β-damascone	0.141 ± 0.002	0.0141 ± 0.0002

Table 3 (*Continued*)

E-liquid sample	Compound detected	Concentration found [mg/mL ± SD (n = 3)]	Concentration found [%w/v ± SD (n = 3)]
	2,6-dimethyklpyridine	0.040 ± 0.001	0.0040 ± 0.0001
	Pyridine	(0.0020)[a]	(0.00020)[a]
Standard_2[b]	Methylcyclopentenolone	6.7 ± 0.2	0.67 ± 0.02
	Ethyl maltol	1.06 ± 0.07	0.1057 ± 0.0075
	β −damascone	0.32 ± 0.01	0.032 ± 0.001
	2,6-dimethylpyridine	0.057 ± 0.005	0.0057 ± 0.0005
	Ethylacetoacetate	0.018 ± 0.002	0.0018 ± 0.0002
	Vanilin	(0.00071)[a]	(0.000071)[a]
	Ionone (α+β)	0.198 ± 0.006	0.0198 ± 0.0006
Banana	Nicotine	18.6 ± 0.8	1.86 ± 0.08
	Linalol	0.149 ± 0.004	0.0149 ± 0.0004
	Vanilin	0.00058 ± 0.00003	0.000058 ± 0.000003
	β-damascone	0.00176 ± 0.00008	0.000176 ± 0.000008
	Nerol + geraniol	0.14 ± 0.01	0.014 ± 0.001
	Ethyl maltol	0.00155 ± 0.00009	0.000155 ± 0.000009
	Linalool oxide	0.013 ± 0.001	0.0013 ± 0.0001
	4-methylacetophenone	0.037 ± 0.003	0.0037 ± 0.0003
	Carvone	0.018 ± 0.001	0.0018 ± 0.0001
	2-acethylpyrrole	0.00057 ± 0.00006	0.000057 ± 0.000006
Black tea	Nicotine	18.3 ± 0.5	1.83 ± 0.05
	Linalol	0.0039 ± 0.0003	0.00039 ± 0.00003
	Nerol + geraniol	0.45 ± 0.02	0.045 ± 0.002
	Linalool oxide	0.053 ± 0.002	0.0053 ± 0.0002
	β-damascone	0.032 ± 0.001	0.0032 ± 0.0001
	Methylsalicylate	0.076 ± 0.002	0.0076 ± 0.0002
	Ethyl phenylacetate	2.6 ± 0.2	0.26 ± 0.02
	Methylcyclopentenolone	0.004 ± 0.002	0.0004 ± 0.0002
Ice mint	Nicotine	18.2 ± 0.5	1.82 ± 0.05
	Menthone	0.11 ± 0.01	0.011 ± 0.001
	Menthol	9.7 ± 0.5	0.97 ± 0.05
	Carvone	0.110 ± 0.004	0.0110 ± 0.0004
	Methylcyclopentenolone	0.003 ± 0.002	0.0003 ± 0.0002
	Linalol	0.0144 ± 0.0008	0.00144 ± 0.00008
	Ethyl maltol	0.086 ± 0.004	0.0086 ± 0.0004
Cappuccino	Nicotine	18 ± 1	1.8 ± 0.1
	2,5-dimethylpyrazine	0.011 ± 0.003	0.0011 ± 0.0003
	2-methylpyrazine	0.0451 ± 0.0006	0.00451 ± 0.00006
	Ethylphenylacetate	0.031 ± 0.002	0.0031 ± 0.0002
	Ethyl maltol	1.38 ± 0.05	0.138 ± 0.005
	Methylcyclopentenolone	18 ± 1	1.8 ± 0.1
	5-methylfurfural	0.2239 ± 0.0008	0.02239 ± 0.00008
	Pyridine	0.0057 ± 0.0002	0.00057 ± 0.00002
	Vanilin	0.22 ± 0.02	0.022 ± 0.002
	Ethylvanilin	0.043 ± 0.001	0.0043 ± 0.0001
	2,3,5-trimethylpyrazine	0.054 ± 0.003	0.0054 ± 0.0003
	2,6-dimethylpyridine	(0.0017)[a]	(0.00017)[a]
Tobacco	Nicotine	18.8 ± 0.9	1.88 ± 0.09
	2-acethylpyridine	0.0099 ± 0.0004	0.00099 ± 0.00004
	Ethyl maltol	0.31 ± 0.04	0.031 ± 0.004
	Methylcyclopentenolone	5.4 ± 0.4	0.54 ± 0.04
	β-damascone	0.051 ± 0.002	0.0051 ± 0.0002
Strong mint	Nicotine	18.1 ± 0.4	1.81 ± 0.04
	Carvone	0.52 ± 0.09	0.052 ± 0.009
	Menthol	13.4 ± 0.8	1.34 ± 0.08
	Menthone	0.34 ± 0.01	0.034 ± 0.001
	Methylcyclopentenolone	3.2 ± 0.1	0.32 ± 0.01
	Linalol	0.054 ± 0.003	0.0054 ± 0.0003
	Ethylvanilin	0.0088 ± 0.0007	0.00088 ± 0.00007
	Linalool oxide	0.0040 ± 0.0002	0.00040 ± 0.00002
	Methylsalicylate	0.023 ± 0.004	0.0023 ± 0.0004
Menthol_1	Carvone	0.19 ± 0.05	0.019 ± 0.005
	Ethyl maltol	0.2232 ± 0.0007	0.02232 ± 0.00007
	Menthol	6.0 ± 0.5	0.60 ± 0.05
	Menthone	0.18 ± 0.01	0.018 ± 0.001
	Ethylphenylacetate	0.024 ± 0.001	0.0024 ± 0.0001
	Linalool	0.096 ± 0.002	0.0096 ± 0.0002
	Nerol + geraniol	0.262 ± 0.001	0.0262 ± 0.0001
	Ethylvanilin	0.170 ± 0.002	0.0170 ± 0.0002
	Linalol oxide	0.013 ± 0.001	0.0013 ± 0.0001
Menthol_2[b]	Nicotine	18.1± 0.3	1.81± 0.03
	Carvone	0.48 ± 0.05	0.048 ± 0.005
	Ethyl maltol	0.330 ± 0.007	0.0330 ± 0.0007
	Menthol	8.1 ± 0.3	0.81 ± 0.03
	Menthone	2.05 ± 0.07	0.205 ± 0.007
	Linalol	0.0024 ± 0.0002	0.00024 ± 0.00002
	Nerol + geraniol	0.79 ± 0.01	0.079 ± 0.001

Table 3 (*Continued*)

E-liquid sample	Compound detected	Concentration found [mg/mL ± SD (n = 3)]	Concentration found [%w/v ± SD (n = 3)]
	Ethylvanilin	0.0068 ± 0.0005	0.00068 ± 0.00005
	Methylsalicylate	0.016 ± 0.001	0.0016 ± 0.0001
	Linalool oxide	0.0070 ± 0.0006	0.00070 ± 0.00006
	Vanilin	0.0017 ± 0.0001	0.00017 ± 0.00001
Peach	Nicotine	16.6 ± 0.9	1.66 ± 0.09
	2-isopropyl-4-methylthiazole	0.071 ± 0.002	0.0071 ± 0.0002
	Linalol	0.00390 ± 0.00008	0.000390 ± 0.000008
	Methylheptenone	0.034 ± 0.002	0.0034 ± 0.0002
	γ – hexalactone	0.019 ± 0.001	0.0019 ± 0.0001
Watermelon	Nicotine	18.4 ± 0.7	1.84 ± 0.07
	Ethyl maltol	0.166 ± 0.003	0.0166 ± 0.0003
	Melonal	0.084 ± 0.004	0.0084 ± 0.0004
	Ethylvanilin	0.045 ± 0.002	0.0045 ± 0.0002
	Diethyl succinate	0.00313 ± 0.00006	0.000313 ± 0.000006
	Nerol + geraniol	0.065 ± 0.001	0.0065 ± 0.0001
	Methylheptenone	0.0245 ± 0.0002	0.00245 ± 0.00002
	Linalol	0.011 ± 0.001	0.0011 ± 0.0001
Cherry	Nicotine	18.2 ± 0.7	1.82 ± 0.07
	Vanilin	0.312 ± 0.008	0.0312 ± 0.0008
	Ionone α + β	0.127 ± 0.002	0.0127 ± 0.0002
	β-damascone	0.0055 ± 0.0001	0.00055 ± 0.00001
	Ethyl maltol	0.0031 ± 0.0001	0.00031 ± 0.00001
	Menthol	0.052 ± 0.009	0.0052 ± 0.0009
Lemon	Nicotine	16.2 ± 0.1	1.62 ± 0.01
	Linalol	0.0084 ± 0.0008	0.00084 ± 0.00008
	Nerol + geraniol	0.057 ± 0.001	0.0057 ± 0.0001
	Carvone	0.017 ± 0.004	0.0017 ± 0.0004
	Vanilin	(0.00072)[a]	(0.000072)[a]
	Maltol	0.00078 ± 0.00002	0.000078 ± 0.000002
Orange	Nicotine	17.47 ± 0.07	1.747 ± 0.007
	Carvone	0.025 ± 0.003	0.0025 ± 0.0003
	Linalol	0.0046 ± 0.0005	0.00046 ± 0.00005
	Nerol + geraniol	0.064 ± 0.007	0.0064 ± 0.0007
	Methylsalicylate	0.0044 ± 0.0004	0.00044 ± 0.00004

[a] value $C_{min} < x < LOQ$.
[b] samples with declared zero-level of nicotine.

Material). The accuracy was in the range of 89–110%, regardless of the spiking level, which indicated that a matrix effect can be neglected during quantification of the analytes. The intra-day precision and the inter-day precision were less than 10%. The precision determined at each concentration level should not exceed 15% of the CV values, except for the lowest level of quantification (LLOQ), where it should not exceed 20% of the CV value [23]. The accuracy of the method should be within the range 80–120%. The precision and accuracy values for the two developed methods were within acceptable ranges, thus, the developed methods proved to be accurate and reproducible.

4. Analysis of real samples

The applicability of the developed method was evaluated by analysing twenty-five e-liquid samples available on the Polish market. The presented analytical protocol is adequate for fast quality control of replacement e-liquids for e-cigarettes. The content of the flavouring compounds and nicotine in the investigated samples are summarized in Table 3. The calculated concentrations that were below the LOD and C_{min} were omitted. Example chromatograms from real samples are presented in Fig. 2. According to Commission Implementing Decision (EU) 2015/2183, which established a common format for the notification of e-cigarettes and refill containers [25], the weight of the ingredients should be expressed in mg in one product unit (mL). To present the results in mg/mL, the density of each liquid was evaluated. For this purpose, 100 μL of each liquid was pipetted using a glass microsyringe onto an analytical scale. The mean density of the investigated e-liquids (n = 25) was 1.130 ± 0.016 g/mL, and this density value was used in further calculations. The results were evaluated as follows: the values analytically determined using the calibration curves (ng/mL) were multiplied by 50 (the volume of the volumetric flask), 10^{-6} (conversion from ng to mg), and 1130 (mean density of the e-liquids in mg/mL) and divided by the weight of the sample (mg). The final results are presented in mg/mL and in% w/v.

The e-liquid samples contain from a few to dozens of flavouring substances, as presented in Table 3. From the 25 e-liquid samples analysed, the lowest total flavour concentration was reported for the e-liquid "Raspberry" at 0.043 mg/mL, and the highest was for "Cappuccino" at 20.1 mg/mL (Fig. S1, Electronic Supplementary Material). The following substances were detected with the highest frequencies in the investigated e-liquids: ethyl maltol (17/25), vanillin (11/25), methyl cyclopentenolone (11/25), ethyl vanillin (7/25), β-damascone (6/25), menthol (6/25) and 2,3,5-trimethylpyrazine (6/25), which agrees with other studies [3,21,26]. 2,3,5-Trimethylpyrazine is a substance typically used for tobacco flavoured e-liquids. In most cases, the measured nicotine concentrations were in accordance with the labelled nicotine levels and equal to 18 mg/mL. Some inconsistencies may be a result of possible nicotine oxidation during manufacturing and storage. Additionally, the nicotine content in e-liquids marked with a zero-level of nicotine was below the LOD.

5. Conclusions

Rapid, sensitive and selective LC–MS/MS methods with minimal sample preparation have been developed and validated for the simultaneous determination of 42 flavouring compounds and nicotine in e-liquids for e-cigarettes. The use of cyclophosphamide as the IS, considerable dilution of the samples, and sufficient separation of the analytes minimized the influence of a matrix effect. The low LOD values, satisfactory accuracy and repeatability make both methods suitable for quality control of e-liquids. To the best

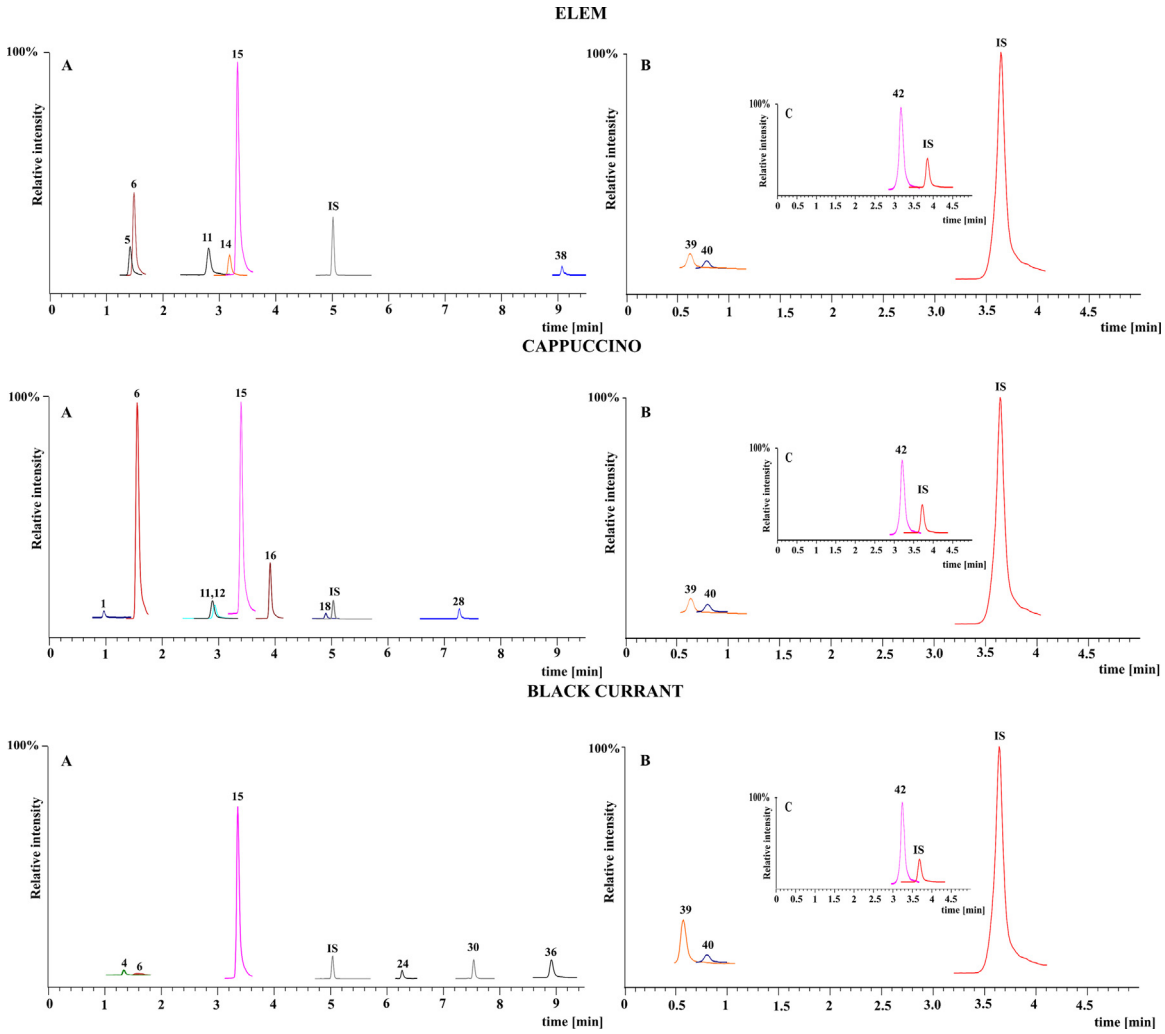

Fig. 2. MRM chromatograms obtained with Ace® Ultracore™ SuperC18™ column (100 × 2.1 mm, 2.5 μm) of real samples. From the top: chromatogram of real sample ELEM, CAPPUCINO and BLACK CURRANT. A – chromatograms obtained at acidic conditions, B- chromatograms obtained at alkaline conditions, C: chromatograms obtained at alkaline conditions after 10 000 x dilution of e-liquid sample to determine nicotine content. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

of the authors' knowledge, these are the first, fully validated methods that can be used to quantify flavouring compounds using a LC–MS/MS technique. Furthermore, the study results indicate the necessity of developing new analytical methods for the surveillance of e-cigarette products to improve the current enforcement of regulations. This study is the first attempt at quantification of multiple flavouring compounds in e-liquids via a LC–MS/MS technique. The presented research will be followed by part II – determination of flavouring compounds via GC–MS. The compounds separated and determined using the method based on a GC–MS technique were not compatible with the LC–MS/MS technique conditions, hence, in such cases, there was a need to look for different tools for determination. The developed LC–MS/MS methods included in Part I together with the GC–MS method described in Part II can be treated as complementary methods to enable multivariate characterization of flavouring compounds in liquids for e-cigarettes.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chroma.2017.08.056.

References

[1] WHO Report on the Global Tobacco Epidemic: Raising Taxes on Tobacco, World Health Organization, 2015 (Accessed 20.05.2017) http://apps.who.int/iris/bitstream/10665/178574/1/9789240694606_eng.pdf.
[2] S. Gravely, G.T. Fong, K.M. Cummings, M. Yan, A.C. Quah, R. Borland, H.H. Yong, S.C. Hitchman, A. McNeill, D. Hammond, J.F. Thrasher, M.C. Willemsen, H.G. Seo, Y. Jiang, T. Cavalcante, C. Perez, M. Omar, K. Hummel, Awareness, trial, and current use of electronic cigarettes in 10 countries: findings from the ITC project, Int. J. Environ. Res. Public Health 11 (2014) 11691–11704.
[3] C. Hutzler, M. Paschke, S. Kruschinski, F. Henkler, J. Hahn, A. Luch, Chemical hazards present in liquids and vapors of electronic cigarettes, Arch. Toxicol. 88 (2014) 1295–1308.
[4] J.L. Pearson, A. Richardson, R.S. Niaura, D.M. Vallone, D.B. Abrams, E-cigarette awareness use, and harm perceptions in US adult, Am. J. Public Health 102 (2012) 1758–1766.
[5] C.I. Vardavas, F.T. Filippidis, I.T. Agaku, Determinants and prevalence of e-cigarette use throughout the European Union: a secondary analysis of 26 566 youth and adults from 27 Countries, Tob. Control 385 (2014) 1–7.
[6] P. Kubica, A. Wasik, A. Kot-Wasik, J. Namieśnik, An evaluation of sucrose as a possible contaminant in e-liquids for electronic cigarettes by hydrophilic

interaction liquid chromatography –tandem mass spectrometry, Anal. Bioanal. Chem. 406 (2014) 3013–3018.
[7] K.E. Farsalinos, R. Polosa, Safety evaluation and risk assessment of electronic cigarettes as tobacco cigarette substitutes: a systematic review, Ther. Adv. Drug Saf. 5 (2014) 67–86.
[8] P. Kubica, A. Kot-Wasik, A. Wasik, J. Namieśnik, Dilute & Shoot approach for rapid determination of trace amounts of nicotine in zero-level e-liquids by reversed phase liquid chromatography and hydrophilic interactions liquid chromatography coupled with tandem mass spectrometry-electrospray ionization, J. Chromatogr. A 1289 (2013) 13–18.
[9] K.E. Farsalinos, K.A. Kistler, G. Gillman, V. Voudris, Evaluation of electronic cigarette liquids and aerosol for the presence of selected inhalation toxins, Nicotine Tob. Res. 17 (2015) 168–174.
[10] M. Manigrasso, G. Buonanno, L. Stabile, L. Morawska, P. Avino, Particle doses in the pulmonary lobes of electronic and conventional cigarette users, Environ. Pollut. 202 (2015) 24–31.
[11] M. Manigrasso, G. Buonanno, F.C. Fuoco, L. Stabile, P. Avino, Aerosol deposition doses in the human respiratory tree of electronic cigarette smokers, Environ. Pollut. 196 (2015) 257–267.
[12] S. Zhu, J.Y. Sun, E. Bonnevie, S.E. Cummins, A. Gamst, L. Yin, M. Lee, Four hundred and sixty brands of e-cigarettes and counting: implications for product regulation, Tob. Control 23 (Suppl 3) (2014) 3–9.
[13] S. Costigan, C. Meredith, An approach to ingredient screening and toxicological risk assessment of flavours in e-liquids, Regul. Toxicol. Pharmacol. 72 (2015) 361–369.
[14] E-Cigarette Use Among Youth and Young Adults: A Report of the Surgeon General, U.S. Department of Health and Human Services, 2016 (Accessed 20.06.2017) https://e-cigarettes.surgeongeneral.gov/documents/2016_sgr_full_report_non-508. pdf.
[15] V. Bansal, K.H. Kim, Review on quantitation methods for hazardous pollutants released by e-cigarette (EC) smoking, TrAC (2016) 120–133.
[16] J.L. Barrington-Trimis, J.M. Samet, R. McConnell, Flavorings in electronic cigarettes: an unrecognized respiratory health hazard, JAMA 312 (2014) 2493–2494.
[17] J.F. Bertholon, M.H. Becquemin, I. Annesi-Maesano, B. Dautzenberg, Electronic cigarettes: a short review, Respiration 86 (2013) 433–438.
[18] L. Kosmider, A. Sobczak, A. Prokopowicz, J. Kurek, M. Zaciera, J. Knysak, D. Smith, M.L. Goniewicz, Cherry-Flavoured electronic cigarettes expose users to the inhalation irritant, benzaldehyde, Thorax 71 (2017) 376–377.
[19] R.Z. Behar, B. Davis, Y. Wang, V. Bahl, S. Lin, P. Talbot, Identification of toxicants in cinnamon-flavored electronic cigarette refill fluids, Toxicol. in Vitro 28 (2014) 198–208.
[20] J.G. Lisko, H. Tran, S.B. Stanfill, B.C. Blount, C.H. Watson, Chemical composition and evaluation of nicotine tobacco alkaloids, pH, and selected flavors in e-cigarette cartridges and refill solutions, Nicotine Tob. Res. 17 (2015) 1270–1278.
[21] M.P. Kavvalakis, P.D. Stivaktakis, M.N. Tzatzarakis, D. Kouretas, J. Liesivuori, A.K. Alegakis, D. Vynias, A.M. Tsatsakis, Multicomponent analysis of replacement liquids of electronic cigarettes using chromatographic techniques, J. Anal. Toxicol. 39 (2015) 262–269.
[22] C.C. Chan, H. Lam, Y.C. Lee, X.-M. Zhang, Analytical Method Validation and Instrument Performance Verification, John Wiley & Sons, Hoboken, 2004, pp. 173–196 (27–66).
[23] Guidance for Industry Q2 B Validation of Analytical Procedures: Methodology (Accessed 20.06.2017) https://www.fda.gov/downloads/drugs/guidances/ucm073384. pdf.
[24] Commission Decision 2002/657/EC of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results, OJEC, 2002, pp. 8–36 (L221).
[25] Commission Implementing Decision (EU)2015/2183 of 24 November 2015 Establishing a Common Format for the Notification of Electronic Cigarettes and Refill Containers, OJEC, 2015, pp. 15–27 (L309).
[26] P.A. Tierney, C.D. Karpinski, J.E. Brown, W. Luo, J.F. Pankow, Flavour chemicals in electronic cigarette fluids, Tob. Control 25 (2015) 10–15.