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Low-field NMR spectroscopic study of e-cigarettes: Is determination of only nicotine and organic carrier solvents possible?

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ABSTRACT

Electronic cigarettes (e-cigarettes) have become popular worldwide with the market growing exponentially in some countries. The absence of product standards and safety regulations requires urgent development of analytical methodologies for the holistic control of the growing diversity of such products. An approach based on low-field nuclear magnetic resonance (LF-NMR) at 80 MHz is presented for the simultaneous determination of key parameters: carrier solvents (vegetable glycerine (VG), propylene glycol (PG) and water), total nicotine as well as free-base nicotine fraction. Moreover, qualitative and quantitative determination of fourteen weak organic acids deliberately added to enhance sensory characteristics of e-cigarettes was possible. In most cases these parameters can be rapidly and conveniently determined without using any sample manipulation such as dilution, extraction or derivatization steps. The method was applied for 37 authentic e-cigarettes samples. In particular, eight different organic acids with the content up to 56 mg/mL were detected. Due to its simplicity, the method can be used in routine regulatory control as well as to study release behaviour of nicotine and other e-cigarettes constituents in different products.

1. Introduction

Electronic cigarettes (e-cigarettes or e-liquids) are advertised as a safer alternative to tobacco consumption [1]. However, the short-term and long-term health risks associated with vaping of any kinds remain still underinvestigated [1–6]. Since their release, e-cigarettes gained big popularity that continues to rise, especially among young people, also due to emergence of new product categories such as “nicotine salts” or “tobacco-free” products [3,5,6].

Given these trends, convenient methods are needed to holistically characterize e-cigarettes composition in existing and new product categories. The main carrier solvents in the e-liquid are vegetable glycerine (VG), propylene glycol (PG) and water, which are mixed in different proportions. Several studies noticed that the labelled and the actual content of these compounds were not always consistent [7,8]. Labelled nicotine content has to be controlled, also regarding maximum nicotine level 20 mg/ml set by the European Union Tobacco Products Directive [9]. Attention should be also given to the nicotine form (free-base or monoprotonated nicotine). Moreover, the type and amount of organic acids should be analysed, because the acid component can have other thermal breakdown products and, therefore, other toxicity than conventional e-cigarettes [5,7].

Reported analytical methods for e-cigarettes analysis were predominantly based on gas chromatography (GC), high-performance liquid chromatography (HPLC) and high-field nuclear magnetic resonance (HF-NMR) spectroscopy [4,7,8,10]. However, these methods have not been used to simultaneously determine the amount of water, PG, VG, nicotine and its form as well as added acid component without sample preparation in a single analytical run.

Benchtop NMR spectroscopy at 40–100 MHz (also called low-field NMR, LF-NMR) using permanent magnets provided an appealing alternative to above-mentioned conventional analytical methods. Contrary to LF NMR relaxometry spectrometers operating at ~2–20 MHz, which is used to characterize the dynamic and diffusion properties of samples [11], Benchtop NMR spectrometers can measure high-resolution NMR spectra providing chemical information about complex matrices. Since a couple of years, such devices are successfully used in a broad variety of areas including reaction and bioprocess monitoring, medicinal diagnostics and analysis of natural, food and pharmaceutical products (see recent reviews [12–14]). Benchtop cryogen-free LF-NMR technology

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proposes cheaper and user-friendly HF-NMR alternative for analytical laboratories, but its possibilities still remain unexplored. The main advantage of LF-NMR considering of e-liquids is that the analysis can be done without any sample manipulation, which may alter acid-base equilibrium of nicotine. Up to now, LF-NMR was only introduced for qualitative analysis of fluorinated synthetic cannabinoids in e-cigarettes [15].

2. Materials and methods

2.1. Samples and chemicals

In total, thirty seven electronic cigarettes in form of refill fluids and disposable e-cigarettes were analysed. Table 1 contained information about investigated samples and labelling information. The samples were bought in local stores in Germany and online shops in 2022–2023. The samples S1-S3, S7-S11 and S28-S36 were provided by the governmental chemical and veterinary agency (Karlsruhe, Germany) in 2021.

L-nicotine (>99 %) was bought from Thermo Scientific Chemicals (Waltham, Massachusetts, USA). Propylene glycol (PG, >99.5 %) was purchased from Sigma-Aldrich (Taufkirchen, Germany). Vegetable glycerol, VG (>99 %) was obtained from Janssen Chimica (Neuss, Germany). NaOH pellets (>98 %) and organic acids – acetic (>99 %), benzoic (>99.5 %), butyric (>99 %), citric (>99.5 %), formic (>98 %), lactic (90 % in water), malic (>99 %), propionic (>99.5 %), octanoic (>99.5 %), salicylic (>99 %), sorbic (>99 %), succinic (>99 %), tartaric (>99.5 %) and valeric (>98 %) – were purchased form Carl Roth (Karlsruhe, Germany). Isopropanol (99.8 %), *tert*-butyl methyl ether (<99.5 %) and NaOD (40 % in D_2O) were also provided by Carl Roth (Karlsruhe, Germany). $CDCl_3$ (D 99.8 %) was provided by Deutero (Kastellaun, Germany). Ethylene glycol was purchased from Honeywell (Charlotte, North Carolina, USA). Diethylene glycol (99 %), 1,3-butanediol and 1,3-propanediol were obtained from Alfa Aesar (Haverhill, Massachusetts, USA).

2.2. Sample preparation for LF-NMR measurements at 80 MHz

600 µL of a sample was then transferred in an NMR tube for analysis. Calibration solutions for nicotine (2.5–25 mg/mL) were prepared in PG/VG mixture (50/50 v/v%). For liquid–liquid extraction 3 mL of an e-cigarette was mixed with 1 mL 2 M NaOH solution in water and 2 mL *tert*-butyl methyl ether. The mixture was thoroughly shaken for 30 Min at an Orbital Shaker Advanced 3500 (VWR, Radnor, Pennsylvania, USA). After separation, the organic phase was evaporated at room temperature and the residual was dissolved in 1 mL $CDCl_3$ for measurement. Aqueous phase was measured directly.

To examine the performance of LF-NMR to identify organic acids in e-cigarettes, synthetic mixtures were prepared. Fourteen organic acids commonly used for e-cigarettes production were investigated [16]. Approximately 70 mg of nicotine and equimolar content of an organic acid (acetic, benzoic, butyric, citric, formic, lactic, malic, octanoic, propionic, salicylic, sorbic, succinic, tartaric or valeric) were first dissolved in 3 mL of PG-VG mixture (50/50 v/v%). To some mixtures 0.5 mL water or/and 0.5 mL PG was added to ensure complete solubility of organic acids. All mixtures were measured directly. Moreover, after extraction according to the above-mentioned procedure both aqueous and organic phases were investigated. For qNMR analysis approximately 10 mg of maleic acid was added to aqueous phase or directly to neat sample as internal standard.

2.3. NMR measurements at 80 MHz

Benchtop NMR measurements at 80 MHz were performed on a Spinsolve 80 Carbon 80 MHz spectrometer equipped with automatic sample changer for twenty samples (Magritek GmbH, Aachen, Germany). The data were recorded automatically under the control of Spinsolve software 14.2.1 (Magritek GmbH, Aachen, Germany).

For PG, VG and water determination, ^{1}H NMR spectra were recorded with the ^{13}C-satellite suppressed ^{1}H NMR experiment with an acquisition time (AQ) of 3.2 s, repetition time (RT) of 30 s, 128 scans (NS), 16 k points in time domain (TD) of and a pulse angle (PA) of 90°. This protocol was also used for the measurement of organic phase after liquid–liquid extraction.

For all other measurements the second experiment was used. ^{13}C-

Table 1
Information about investigated e-cigarettes and labelled parameters.

Sample	Type of product[a]	Nicotine content [mg/mL]	Carrier solvent composition	Flavorings
S1	A	8	GL/PG	Menthol
S2	A	6	GL/PG	Wild berry flavor
S3	A	3	GL/PG	Menthol
S4	A	may contain traces of nicotine	GL/PL (50/50)	Mentha flavor
S5[d]	B	20 (nicotine salts)	GL/PG	Peach/Mango/ Watermelon flavor
S6 [d]	B	20	GL/PG	Menthol
S7	A	6	GL/PG/Water (38/50/12)	Tobacco flavor
S8	A	12	GL/PG	Tobacco flavor
S9	A	12	GL/PG	Citrus fruit flavor Red pomegranate flavourTequila flavor
S10	A	12	GL/PG	Cherry flavor
S11	A	3	GL/PG/Water (60/30/10)	Chocolate flavor
S12	A	20 (nicotine salts)	GL/PG (50/50)	Banane flavor
S13	A	20 (nicotine salts)	GL/PL (50/50)	Peach/Mango flavor
S14	A	20 (nicotine salts)	GL/PL (50/50)	Kiwi/cactus flavor
S15	A	20 (nicotine salts)	GL/PL (50/50)	Cherry flavor
S16	A	12	GL/PL	Flavors
S17	B	20 (nicotine salts)	GL/PG	Cola/Vanille flavor
S18	A	NF[b]	GL/PG	Mentha flavor
S19	A	NF	GL/PG	Berry flavor
S20	A	NF	GL/PG	Peach/Passion fruit flavor
S21	A	16	GL/PG	Peach/Passion fruit flavor
S22	A	NF	GL/PG	Tobacco flavor
S23	A	8	GL/PG	Tobacco flavor
S24	A	NF	GL/PG	Strawberry flavor
S25	A	16	GL/PG	Strawberry flavor
S26	A	16	GL/PG	Tobacco flavor
S27	A	NF	GL/PG	–[c]
S28	A	3	GL/PG	– [c]
S29	A	12	GL/PG	Menthol
S30	A	12	GL/PL (50/50)	– [c]
S31	A	9	GL/PL/Water (35/55/10)	– [c]
S32	A	18	GL/PG	– [c]
S33	A	3	GL/PG	– [c]
S34	A	18	GL/PL (>30/ >45); Water	– [c]
S35	A	3	GL/PL (50/50); Water	– [c]
S36	A	6	GL/PG	– [c]
S37	B	50	GL/PG	– [c]
S38	B	50	GL/PG	– [c]
S39	B	50	GL/PG	– [c]

[a] Refill bottle (A)/Disposable E-Cigarette (B).
[b] – NF – nicotine free.
[c] No flavours were declared by the producer.
[d] The samples were destroyed before analysis.

satellite suppressed ^{1}H NMR spectra were recorded with the simultaneous solvent suppression of three signals (5.5–4.0 ppm, 3.9–3.0 ppm, 2.0–1.0 ppm) and an AQ of 3.2 s, RT of 30 s, NS 512, and a PA of 90°. Spin–lattice relaxation times (T1) were determined using inversion-recovery experiment in aqueous and organic phases. The results showed that the RT of 30 s was enough for quantitative determination.

2.4. NMR spectra processing and quantitative analysis

NMR spectra were manually processed using Mestrenova 14.2.3 (Mestrelab Research S.L., Santiago de Compostela, Spain). All spectra were first phase- and baseline corrected. Exponential apodization function of 0.2 Hz was applied to free induction decay (FID). Zero filling was set to double of a particular TD value. Integration was performed by summation of all points under a peak for undisturbed signals or peak deconvolution for overlapping signals.

PG/VG ratio in v/v% was found by comparing the signal areas at δ 1.3–0.9 ppm (propylene glycol (3H-atoms)) and at δ 4.1–3.0 ppm (propylene glycol (3H-atoms) and glycerine (5H-atoms)). Nicotine was quantified at δ 8.6 ppm by external calibration in the 2.5–75 mg/mL range in PG/VG mixture. Limits of detection (LOD) and quantification (LOQ) for nicotine were determined as 0.1 mg/mL and 0.3 mg/mL in matrix as signal-to-noise ratio (SNR) equal to 3 and 9, respectively. This is the most common way to determine detection limits in NMR spectroscopy [17]. Measurement uncertainty estimated over 5 days was below 0.2 %.

For water quantification, two specific ranges (δ 2.2–1.9 ppm and δ 4.6–2.9 ppm) were integrated and the sum was normalized to 80. This is proportion to the sum of H-atoms in CHx groups in PG and VG. Then the OH-signal at δ 5.7–4.7 ppm was than integrated and the resulted relative value (ranged from 50.8 to 82.0 for authentic samples) was correlated to the water content in e-cigarettes samples found by the reference NIR method. Similar approach was previously used for water determination in heparin samples [18].

The nicotine/organic acid mole ratio was first quantified in neat e-cigarettes samples with known nicotine content. Additionally, qNMR was performed for neat nicotine-free samples and some samples after extraction using MA as internal standard (δ 5.9 ppm, singlet, 2H).

For the determination of free-base nicotine fraction, the method developed for HF-NMR was adopted from [19]. Chemical shifts of free-base nicotine was defined after addition of 20 μL NaOD (40 %). Chemical shifts of monoprotonated nicotine were defined after addition of 170 μL acetic acid, which corresponds to 10-fold mole excess of acetic acid (Fig. S1 in Supplementary material). The following equation was used: $\Delta\delta = (\delta_c - \delta_d) + (\delta_a - \delta_d)$. For signal assignments see Fig. S1 in Supplementary material. For free-base nicotine and monoprotonated samples $\Delta\delta$ values were calculated as 11.73 ppm and 10.88 ppm, respectively. Free-base nicotine fraction in the investigated samples was calculated as following:

$$\alpha_{fb} = \frac{(\Delta\delta_{commercialsample} - \Delta\delta_{monoprotonatedstandard})}{(\Delta\delta_{free-basestandard} - \Delta\delta_{monoprotonatedstandard})} * 100$$

Because of signal overlap, only signals c and d were used for the samples with benzoic and salicylic acids.

2.5. Chemometrics

To construct principal components analysis (PCA) model the data points from two spectral regions (δ 1.42–3.14 ppm, δ 6.20–10.50 ppm) pre-processed by bucketing with 0.02 ppm width were submitted to Matlab 2022b (The Math Works, Natick, MA, USA). To normalize the intensities in different samples, buckets were scaled to total intensity. SAISIR package for MATLAB was used for statistical calculations [20].

2.6. Reference GC-FID analysis

GC analysis was performed on a gas chromatograph GC-2010 Plus equipped with an autosampler for ten samples and flame ionization detection (FID) detector (Shimadzu GmbH, Duisburg, Germany). A FS-INNOPEG 2000 (15 m × 0.15 mm × 0.25 μm) column was used (CS-Chromatographie Service GmbH, Langerwehe, Germany). Nitrogen carrier gas was run through a 50:1 split injector at a temperature of 250 °C and a flow rate of 0.71 mL/min (40 cm/s). The oven temperature was set to 100 °C and held for 1 min. Then the temperature was increased by 15 °C/min to 130 °C and subsequently by 40 °C/min to 200 °C and then held for 2 min. Finally, the temperature was increased by 40 °C/min to 260 °C and then held for 5 min. The FID was kept at 275 °C.

For sample preparation approximately 100 mg of each sample was weighed and dissolved up to 10 mL with isopropanol. The sample was then vortexed and 1 mL was transferred into a GC vial.

The chromatograms of authentic samples were first screened regarding the presence of carrier solvents ethylene glycol, 1,3-butanediol, 1,3-propanediol and diethylene glycol. These compounds were not detected in the investigated samples. For quantification of nicotine, PG and VG external calibration was used. Peaks corresponded to VG (t = 6.052 min.), nicotine (t = 4.754 min.) and PG (t = 3.388 min.) were integrated using LabSolutions v. 5.93 (Shimadzu, Germany). The example chromatogram can be found in Fig. S2 in Supplementary material. Table 2 contained absolute and normalized to 100 % VG/PG ratios in v/v%. The method was successfully validated in-house regarding precision, linear range, recovery rate, and LOD/LOQ (see Table S1 in Supplementary material).

2.7. Water determination by near-infrared (NIR) spectroscopy

NIR-spectra were acquired on a NIR spectrometer Vector 22/N (Bruker, Ettlingen, Germany). The spectral range of the instrument was 4000–10000 nm and the resolution was 8 cm^{-1}. 32 scans were recorded in diffuse reflection by placing a quartz vial with a sample on the integration sphere of the spectrometer. Each sample was measured in triplicate. The spectra were averaged and pre-processed by standard normal variate (SNV). Several calibration solutions were prepared that contained distilled water from 1 v/v% to 10 v/v % (1 %, 3 %, 5 %, 7 %, and 10 %) in PG/VG 50/50 v/v% mixture. Water content was determined by external calibration method at 5200 cm^{-1} (Fig. S3 in Supplementary material).

3. Results and discussion

3.1. NMR determination of organic carrier solvents: PG and VG

Fig. 1 showed the ^{1}H NMR spectrum of a neat e-cigarette sample as well as a magnification of the δ 9.5–7.0 ppm region, where aromatic signals of nicotine were observed. Contrary to HF-NMR profile [8], PG and VG signals overlapped in the δ 4.0–3.0 ppm region.

According to the reference GC-FID analysis, VG and PG were predominant components in e-cigarettes with the average sum total and minimum contents of 93 v/v % and 81 v/v%, respectively (Table 2). The 80 MHz NMR results for VG/PG volume ratio without any calibration corresponded to the existing labelling information for eleven products (Table 2). Similar analytical ranges for e-cigarettes contained different flavourings were found by NIR spectroscopy: 25–55 v/v % and 35–65 v/v % for PG and VG, respectively [21]. LF-NMR results were also comparable with the normalized GC-FID results. Similarly, the presence and the contents of PG and VG determined by other methods generally corresponded to the labelling [8,21–23]. Our NMR calibration-free approach based on LF-NMR equipment seemed to be suitable for estimation of carrier solvent composition in e-cigarettes.

Table 2
Sample composition determined by GC-FID, NIR and NMR.

Sample	Total nicotine [mg/mL]			VG/PG ratio [v/v %]				Water content NIR [vol.%]
	GC-FID	NMR	Labelled	GC-FID	GC-FID[c]	NMR[c]	Labelled[d]	
S1	7.3	9.5	8	25/75	26/74	30/70		n.d.[a]
S2	5.8	6.4	6	26/74	26/74	25/75		n.d.
S3	2.7	2.9	3	25/68	27/73	26/74		0.9
S4	26.5	23.5	traces	55/31	64/36	55/45	50/50	0.7
S7	5.0	6.3	6	32/55	37/63	36/64	38/50	11.3
S8	9.9	10.6	12	50/42	54/46	51/49		3.3
S9	Peak overlap	10.3	12	53/45	54/46	51/49		4.0
S10	12.0	12.7	12	52/45	64/46	50/50		3.6
S11	3.4	3.0	3	62/34	64/35	60/40	60/30	11.0
S12	22.6	21.4	20	57/43	56/44	51/39	50/50	n.d.
S13	18.4	16.4	20	58/36	62/38	55/45	50/50	n.d.
S14	27.5	29.3	20	55/41	57/43	53/47	50/50	1.0
S15	26.6	21.3	20	62/38	61/39	53/47	50/50	1.0
S16	11.4	11.2	12	48/49	49/51	46/54		6.4
S17	–[b]	22.8	20	–[b]	–[b]	54/46		–[b]
S18	n.d.	n.d.	20	23/66	26/74	24/76		5.6
S19	n.d.	n.d.	NF[e]	24/71	25/75	25/75		5.9
S20	n.d.	n.d.	NF	24/67	26/74	24/76		1.4
S21	14.7	19.7	NF	24/67	26/74	27/73		5.5
S22	n.d.	n.d.	16	25/75	25/75	25/75		n.d.
S23	7.5	9.5	NF	8/86	9/91	9/91		n.d.
S24	n.d.	n.d.	8	24/75	24/76	23/77		n.d.
S25	15.3	16.1	NF	23/66	26/74	25/75		n.d.
S26	16.2	18.8	16	25/73	26/74	26/74		n.d.
S27	n.d.	n.d.	16	23/68	25/75	24/76		0.9
S28	3.4	3.0	NF	23/70	25/75	26/74		4.3
S29	11.5	11.6	12	10/74	12/88	11/89		13.4
S30	10.1	9.9	12	42/40	51/49	51/49	50/50	11.4
S31	9.6	9.9	9	30/58	34/66	32/68	35/55	13.1
S32	17.3	15.1	18	44/50	47/53	47/53		5.7
S33	3.1	2.9	3	49/32	60/40	56/44		11.4
S34	17.5	14.7	18	30/52	37/63	36/64	>30/>45	12.9
S35	3.2	2.5	3	46/47	49/51	49/51	50/50	8.8
S36	6.1	5.4	6	32/57	36/64	35/65		8.9
S37	–[b]	53.6	50	–[b]	–[b]	52/38		–[b]
S38	–[b]	59.7	50	–[b]	–[b]	52/38		–[b]
S39	–[b]	51.8	50	–[b]	–[b]	50/50		–[b]

[a] n.d – not detected (LOD for nicotine were 0.1 mg/mL and 0.02 mg/mL for NMR and GC, respectively; LOD for H_2O was 0.7 vol% by NIR).
[b] – Was not determined.
[c] – The sum of PG and VG was normalized to 100%.
[d] – For samples, where quantitative information was provided on the label.
[e] – NF – nicotine free.

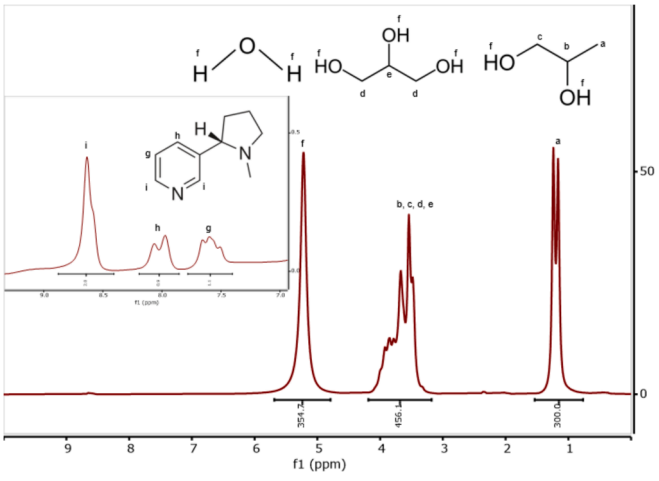

Fig. 1. ^{1}H NMR spectrum of a representative e-cigarette sample showing signals of carrier solvents and nicotine. The spectra were measured using standard ^{1}H protocol without solvent suppression (see experimental section). The signal assignment is shown with letters. The insert shows ^{1}H NMR spectrum in the δ 9.5–7.0 ppm range. Y-axes represent intensity in [A.U.].

3.2. Determination of water by NMR

The idea behind water determination by ^{1}H NMR was that the e-cigarettes predominantly consisted of PG and VG (the sum is nearly 100 %) (Fig. 1). The peak between δ 4.7 and δ 5.7 ppm in the NMR-spectrum represented the sum of exchangeable OH-groups of PG, VG and water. Therefore, a linear correlation should exist between the actual water content and the relative OH-integral. Fig. S4 in Supplementary material showed ^{1}H NMR spectra of two e-cigarettes samples with different water content of 1 v/v % (S3) and 13 v/v % (S34), for which the relative OH-integral values were 50.8 and 74.0, respectively.

The normalized OH-peak areas (ranged from 50.8 to 82.0) were plotted against reference water content values found by NIR method for 27 samples. The calibration equation was determined as $Y = (197 \pm 10) * X + (50 \pm 5)$ with a linear correlation coefficient of 0.95 over a reference range of water content between 0 v/v % and 16 v/v % (Fig. S5 in Supplementary material). The reference range of 0.6–18 v/v % for water was determined for 136 e-liquid samples [21].

Water was quantified in twenty-four samples, but was labelled only in five samples (Tables 1 and 2). High water content in S7, S11, S31, S34 and S35 was in accordance with the labelling. The presence of water in other samples can be explained by the fact that the manufacturers may have used cheap chemicals containing residual water. Another reason could be that VG and PG are highly hygroscopic and water could be

accumulated during storage. Indeed, samples S30, S33 and S36 with high water content were measured ca. 2 years after their production.

Besides PG/VG ratio, water content is the second parameter, which can be determined without any prior sample preparation by LF-NMR. HF-NMR spectroscopy was previously applied to moisture determination in heparin samples [18]. Potentially, this elegant approach for water determination can be applied to other matrices such as honey and dairy powders on both HF- and LF-NMR spectrometers.

3.3. LF-NMR determination of total nicotine content

The peak at δ 8.65 ppm corresponded to two H-atoms was used for total nicotine quantification, because this signal was not interfered in any case (for example, by benzoate used for the production of "nicotine salts") (Fig. 1). The quantitative results for total nicotine content obtained by NMR were summarized in Table 2. The NMR results showed consistency with the reference GC/MS method (slope 0.97 and R^2 = 0.95; Fig. S6 in Supplementary material). The greater discrepancy was found between the actual and the labelled nicotine content. Only two of seven samples declared as "nicotine-free"/"nicotine traces" actually contained nicotine below LOD. In another study 21 % of the investigated e-cigarettes samples that were declared as nicotine-free contained detectable nicotine concentrations [8].

No nicotine was detected in four samples (S18, S22, S24 and S27) although it was labelled by the manufacturer. Samples S37-S39 produced in the USA showed nicotine content considerably higher than the limit set of 20 mg/ml by the European Union Tobacco Products Directive [9]. These inconsistencies indicated that the total nicotine content in e-cigarettes has to be controlled more thoroughly. Benchtop NMR at 80 MHz with the LOD of 0.1 mg/mL for nicotine is not as sensitive as conventional NMR at 400 MHz with LOD 0.02 mg/mL [8]. However, it still represents a good alternative analytical approach for typical contents of nicotine in e-cigarettes (2–60 mg/mL according to the [21]).

3.4. LF-NMR determination of free-base nicotine content

Apart from total nicotine content in e-cigarettes, its form should be controlled. Depending on the composition of e-cigarettes, nicotine in finished products can present as free-base and monoprotonated species in equilibrium [4]. The free-base fraction α_{fb} affects sensory attributes of e-cigarettes, therefore, e-cigarettes with higher nicotine content can be consumed [19]. Commercial labels on e-cigarettes products do not indicate α_{fb} values.

Free-base fractions α_{fb} together with total nicotine content for all investigated commercial e-cigarettes were summarized in Table 3. The percent of free-base nicotine varied between 0 % and 97 %. Similar, α_{fb} values between 3 % and 84 % were found for one e-liquids producer in another study [19]. For the samples with nicotine content below 10 mg/ml, the free-base nicotine form was predominant and varied between 72 % and 93 %. On the contrary, nicotine mainly existed in its monoprotonated form in the samples with the total nicotine content more than 20 mg/mL. It is known that the monoprotonated form has significantly lower throat irritation and slower absorption rate [4]. Three samples S37-S39 with extreme high nicotine content of more than 50 mg/mL contained exclusively monoprotonated nicotine form. Samples with the total nicotine content between 9 and 30 mg/mL can be grouped in clusters: nine samples with $\alpha_{fb} > 80$ %, six samples with $40\ \% < \alpha_{fb} < 80$ % and five samples with $\alpha_{fb} < 40$ %. Apparently, in this concentration range samples with high free-base nicotine fraction still had satisfactory sensory profile.

To characterize nicotine forms in e-cigarettes several methods based on GC–MS [4,24], liquid–liquid extraction [24], non-aqueous pH measurements [25], X-ray photoelectron spectroscopy [26] and HF-NMR spectroscopy [19,27] have been proposed. On the contrary, our LF-NMR approach adopted from Duell et al. [19] is the only method without sample manipulation and, therefore, do not alter acid-base equilibrium of nicotine molecule.

Table 3
Content of nicotine and acidic components in e-cigarettes.

Sample	Nicotine [mg/mL]	Organic acid	Mole ratio Nicotine/acid	Acid content [mg/mL]	Free-base fraction α_{fb} [%]
S1	9.5	–[a]	–	–	92 %
S2	6.4	–	–	–	85 %
S3	2.9	–	–	–	83 %
S4	23.5	Lactic	1.3	10.0	10 %
		Succinic	0.8	21.4	
S7	6.3	–	–	–	88 %
S8	10.6	Butyric	1.1	5.2	96 %
S9	10.3	–	–	–	89 %
S10	12.7	–	–	–	97 %
S11	3.0	Butyric	0.2	8.1	74 %
S12	21.4	Benzoic	1.1	14.6	28 %
		Propionic	0.4	24.4	
S13	16.4	Benzoic	1.4	8.8	34 %
		Propionic	0.5	15.0	
S14	29.3	Lactic	2.0	5.4	42 %
S15	21.3	Salicylic	0.7	25.9	39 %
S16	11.2	–	–	–	94 %
S17	22.8	Salicylic	0.7	27.7	35 %
S18	n.d.	Propionic	–	3.1	n.d.
S19	n.d.	Acetic	–	1.0	n.d.
S20	n.d.	Formic	–	4.1	n.d.
S21	19.7	Butyric	1.1	9.7	77 %
S22	n.d.	Succinic	–	4.9	n.d.
S23	9.5	–	–	–	48 %
S24	n.d.	Butyric	–	4.9	n.d.
S25	16.1	–	–	–	94 %
S26	18.8	–	–	–	96 %
S27	n.d.	–	–	–	n.d.
S28	3.0	Propionic	0.5	1.1	72 %
		Acetic	1.0	2.7	
S29	11.6	–	–	–	97 %
S30	9.9	Butyric	0.40	13.4	64 %
S31	9.9	–	–	–	74 %
S32	15.1	–	–	–	98 %
S33	2.9	Benzoic	1.7	1.3	93 %
S34	14.7	Acetic	1.3	4.2	46 %
S35	2.5	–	–	–	89 %
S36	5.4	Butyric	0.60	4.9	82 %
S37	53.6	Benzoic	0.80	50.4	0 %
S38	59.7	Benzoic	0.80	56.2	3 %
S39	51.8	Benzoic	0.70	55.7	4 %

[a] Any acidic component was detected.

3.5. Determination of organic acids in synthetic mixtures

Since many unassigned signals were observed in ^{1}H-NMR spectra of the investigated samples, the NMR spectra were screened regarding other constituents. First principal component analysis (PCA) was performed for exploratory data analysis. The projections of NMR spectra of nicotine-containing samples on the first three principal components (PCs) were shown in Fig. 2. Several groups of outliers were identified: S37, S38, S39 as well as S12, S13 and S15 had positive scores values along PC1; S17, S30, S33, S34 had negative scores along PC2 and S01, S04, S14 and S17 deviated along PC3. These deviations could originate from the addition of week organic acids, which has become a trend due to increased flavour and greater sensory effects associated with the domination of monoprotonated nicotine form [16]. Indeed, samples S04, S12-S15, and S17 were labelled as "nicotine-salts" products, but the type and the content of organic acid was not specified in any case (Table 1).

To test the possibility of LF-NMR for identification of organic acids added to e-cigarettes, first experiments with synthetic mixtures were carried out. Fig. 3 showed the spectra of synthetic mixtures containing equimolar concentration of nicotine and organic acids in PG/VG binary mixture. From fourteen investigated organic acids used in e-cigarette

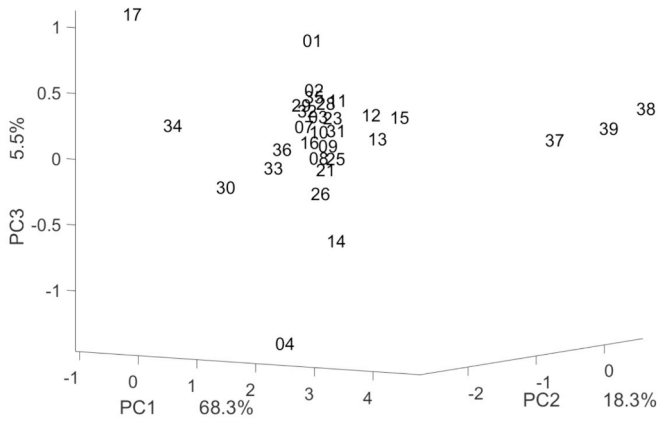

Fig. 2. PCA scatter plot of ^{1}H NMR spectra of nicotine-containing samples. The spectra were measured using solvent suppression in three regions (see experimental section). Sample number correspond to those in Table 1.

production, nine can be identified even from the neat ^{1}H NMR spectrum of synthetic mixtures (Fig. 3). The identification is especially unambiguous for aromatic and unsaturated organic acids such as sorbic, benzoic and salicylic acids, which showed their characteristic signals between δ 5.5 ppm and δ 8.0 ppm. Unsubstituted saturated monocarboxylic acids such as formic, acetic, propionic and butyric acids can be identified at δ 8.8 ppm, δ 2.1 ppm, δ 2.3 ppm, δ 1.7/2.2 ppm, respectively (Fig. 3). Dicarboxylic non-volatile acids, namely tartaric and succinic acids, can be also detected at δ 4.7 ppm and δ 2.6 ppm, respectively (Fig. 3).

The specific signals of other acids were covered by PG and/or VG resonances. After liquid–liquid extraction, the presence of citric, malic and lactic acids can be additionally justified. Totally, twelve of fourteen acids were identified in aqueous phase (Fig. S7 in Supplementary material). Only three monocarboxylic acids (butyric, valeric and octanoic acids) were extracted in organic phase, where only the rest of PG and nicotine signals disturbed the counter ions identification (Fig. S8 in Supplementary material). Butyric acid was the single compound detected in both phases (Figs. S7 and S8).

3.6. Nicotine salt detection in e-cigarettes

Neat ^{1}H- NMR spectra were screened regarding the presence of acidic components. As an example, Fig. 4 showed LF-NMR spectra in the downfield region of e-cigarettes containing formic, benzoic and salicylic acids. Likewise, saturated organic acids such as acetic, butyric, succinic and propionic acids were identified in the upfield region (Fig. 5). Even lactic acid was identified in NMR spectra of two samples S4 and S14. The results were justified by the analysis of aqueous and organic phases after liquid–liquid extraction of e-cigarettes as well as spiking experiments.

The quantitative results for the investigated samples were summarized in Table 3. Twenty-tree products contained one or more acidic components (Table 1, Table 3). Totally, eight organic acids were detected: butyric (six samples), benzoic (five samples), propionic (four samples), acetic (three samples), lactic, succinic, salicylic (each in two samples) and formic (one sample) acids (Table 3). Interestingly, nicotine-free samples S18-S20, S22 and S24 also contained different organic acids, preliminary short-chain fatty acids such as formic, acetic, propionic and butyric. Three samples showed the presence of two acids (S12, S13 and S28).

This is consistent with the results of chromatographic studies, where

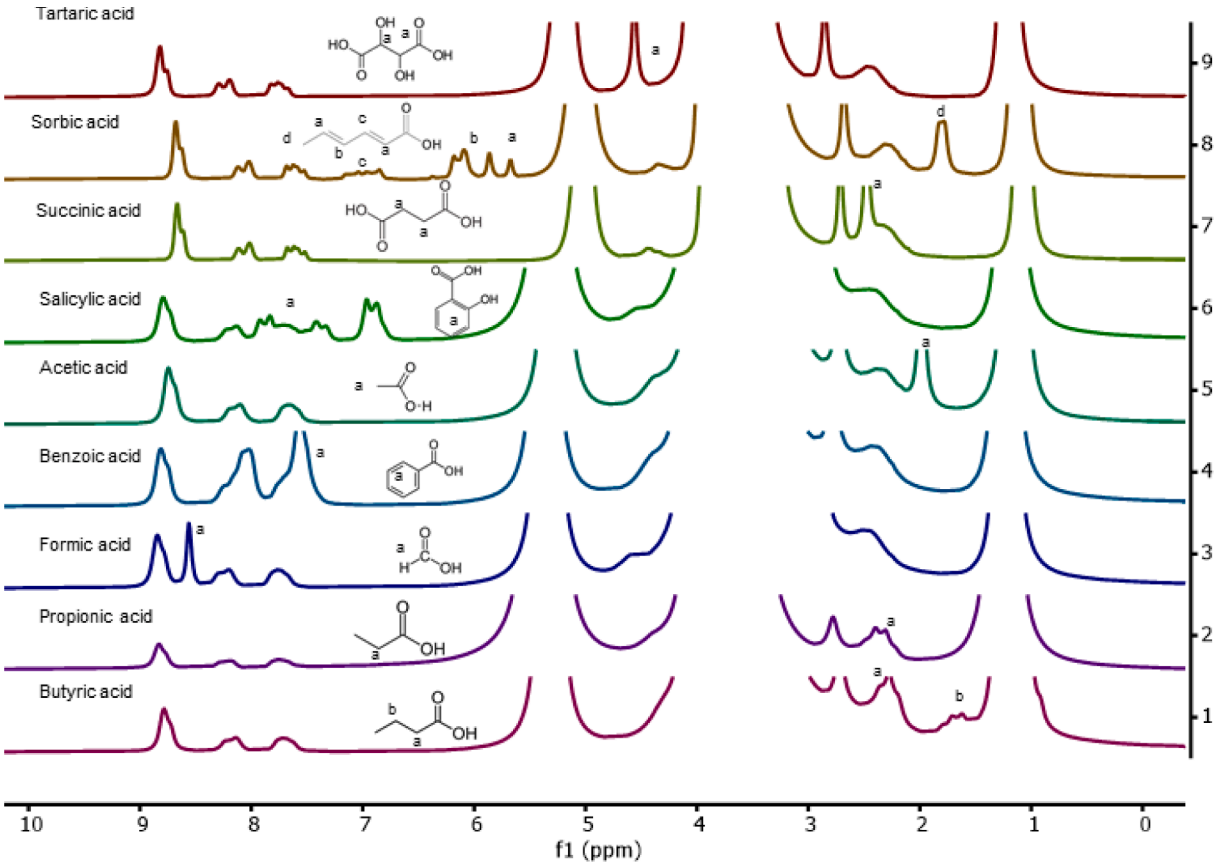

Fig. 3. ^{1}H NMR spectra of synthetic nicotine-organic acid mixtures in PG/VG 50/50 v/v% binary solvent. Only acids, which can be identified in neat mixtures were shown. The spectra were measured using solvent suppression in three regions (see experimental section). The signal assignments were shown with letters. Y-axis represents intensity in [A.U.].

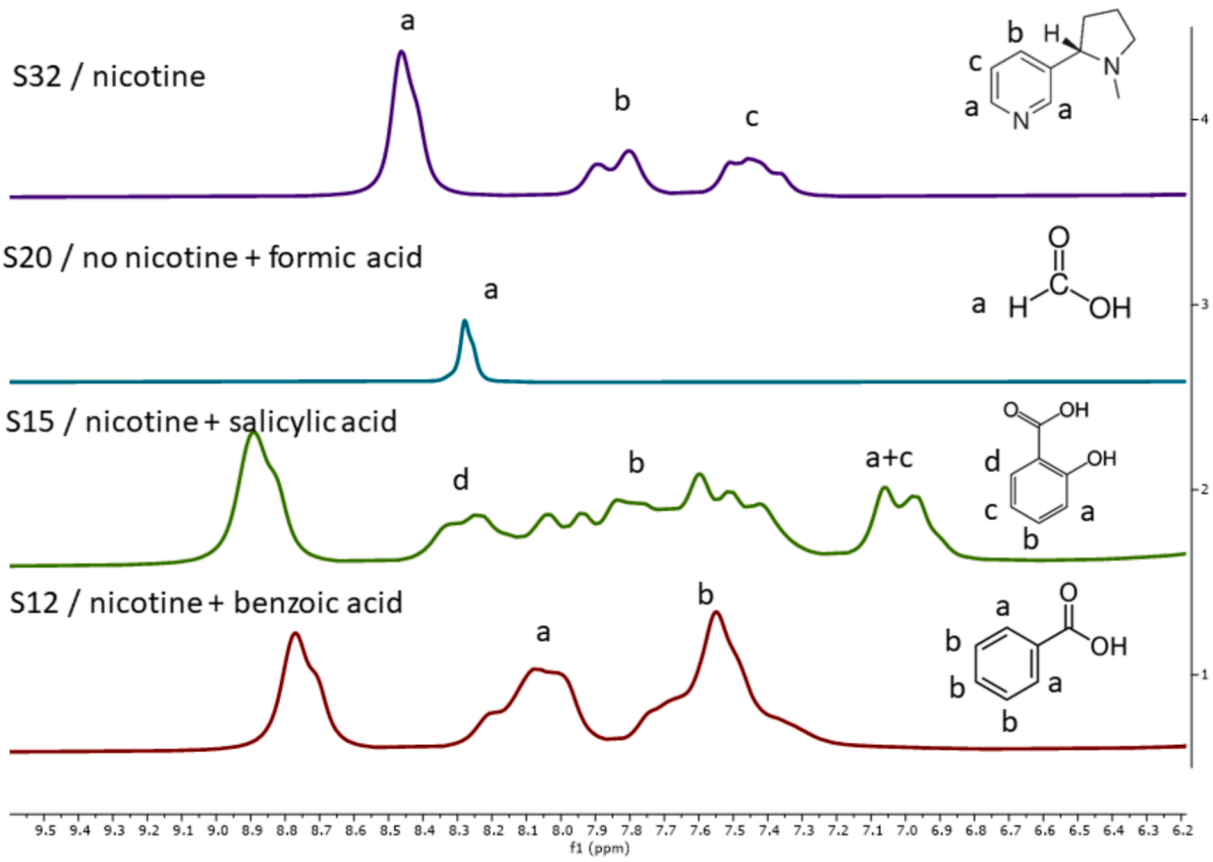

Fig. 4. Neat ^{1}H NMR spectrum of the samples S12, S15 and S20 compared with a "salt-free" sample S32 in the aromatic region. The spectra were measured using solvent suppression in three regions (see experimental section). The signal assignment is shown with letters. Y-axis represents intensity in [A.U.].

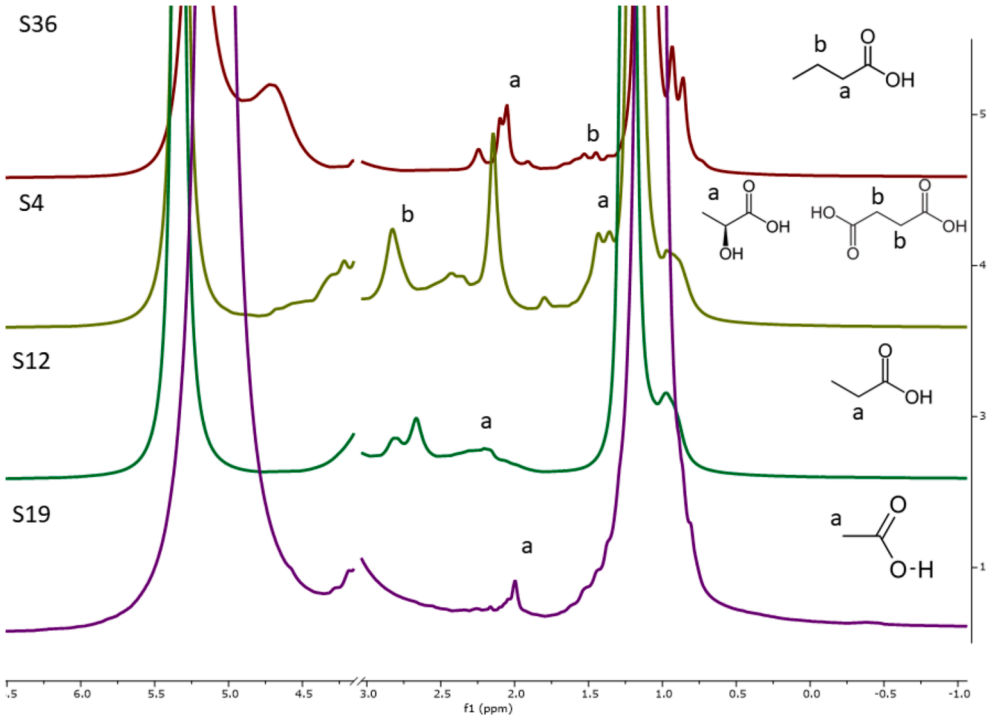

Fig. 5. Neat ^{1}H- NMR spectrum of the samples S4, S12, S19 and S36 in the upfield region. The spectra were measured using solvent suppression in three regions (see experimental section). The signal assignment is shown with letters. Y-axis represents intensity in [A.U.].

benzoic and lactic acids as well as acetic and butyric acids were the most common non-volatile and volatile acids in e-cigarettes, respectively [7,16]. On the contrary, levuliniuc acid, which was identified among the most abundant compounds [7,16], was not detected in our samples. The results suggested that currently the acidic content in e-cigarettes products is not under control and should be surveyed more thoroughly. Recent animal experiments proved different effects on metabolism and neuronal activity of "nicotine salts" and free-base products [28].

The results of manual investigation was connected to the non-targeted PCA findings (Fig. 2, Table 3). The projection of the samples with no acidic component and where nicotine existed predominately in its free-base form occupied the center of the PCA score plot (for example, samples S9, S10, S25, S16). The cluster along positive PC1 values contained samples with benzoic acid (S12, S13, and S37-S39). The S17 contained high amount of salicylic acid also had deviated scores. Samples S4 and S14 with lactic acid and low free-base nicotine fraction deviated along PC3. Therefore, non-targeted approach can be used for the preliminary LF-NMR spectra investigation.

3.7. Quantification of nicotine salts in e-cigarettes

Quantitative NMR results for the content of organic acids expressed as nicotine/acid mole ratio and as mass concentration in mg/mL in e-cigarettes were summarized in Table 3. Mole ratio of nicotine/organic acid ranged between 0.4 (S12, propionic acid) and 2.0 (S14, lactic acid) (Table 3). The results suggested that organic acids were added in e-cigarettes close to, but not in exact stoichiometric ratio to nicotine in most of the cases (average 0.9, median 0.8 for our investigated products). Han et al. also found not stoichiometric molar ratios for main organic acids, for example, for nicotine/benzoic acid the ratio was found between 1:0.63 and 1:1.53 [16]. This means that most products rather had nicotine and an organic acid as separate ingredients than its actual salt [3,16].

The mass concentrations of organic acids ranged from 1.0 mg/mL for acetic acid in S19 to 56.2 mg/mL for benzoic acid in S38. Small volatile organic acids were observed in lower levels: for example, acid content varied between 1.0 and 4.2 mg/mL or 4.9 and 13.4 mg/mL for acetic and butyric acids, respectively (Table 3). In contrast, aromatic benzoic and salicylic acids were present at levels up to 56 mg/mL and 26 mg/mL, respectively. Han et al. detected benzoic acid in the range from 15.1 to 52.3 mg/g [16]. The quantitative results further suggested that free-base nicotine fraction is clearly related to absolute concentration of weak acids but not to nicotine/acid mole ratio (Table 3).

Most of the analytical techniques related to organic acid determination in tobacco products are based on GC–MS with prior derivatization [7] or LC-MS/MS [7,16]. In some approaches cumbersome multi-step sample preparation is required [7]. LODs for non-volatile and volatile organic acids were found in the range 0.002–0.20 mg/g and 0.02–0.2 mg/g, respectively [8]. However, different equipment is required for the determination of volatile, semi-volatile and non-volatile acids [16]. On the contrary, being not so sensitive, our method is suitable for the detection of different organic acids without sample preparation.

4. Conclusion

This study extends our series of studies aimed at the exploration of LF-NMR spectroscopic technique in the context of qualitative and quantitative mixture analysis [29–31]. In this study LF-NMR was used for the simultaneous determination of carrier solvents (VG, PG and water), total nicotine as well as free-base nicotine fraction in e-cigarettes. Moreover, non-targeted PCA of ^{1}H NMR spectra identified samples with added acidic components (which at the same time showed considerably large content of monoprotonated nicotine). The fundings were confirmed by univariate screening of NMR spectra. Eight organic acids (butyric, benzoic, propionic, acetic, lactic, succinic, salicylic and formic) were detected in twenty-tree products out of thirty-seven investigated products. These fundings together with inconsistency of labelled and actual nicotine content in some samples should provoke action towards more careful regulation of e-cigarettes.

Regarding e-cigarettes analysis, in most of the previous studies HF-NMR systems (500–600 MHz) were used, which required the addition of deuterated solvent [8,15,19,22,32]. The research topics included detection of synthetic cannabinoids, flavouring compounds and potentially harmful constituents in aerosols as well as investigation of nicotine protonation in water [2,15,32,33]. E-cigarettes can be analysed without any pre-treatment by LF-NMR spectrometers, which could affect the acid-base equilibrium.

This study opens the possibilities for further research towards NMR spectroscopic investigation of e-cigarettes. First, it would be interesting to qualitatively and quantitatively determine flavouring and preservative profile. Another topic would be to differentiate plant-derived and synthetic nicotine, which is used for the production of novel "tobacco-free" products [6].

CRediT authorship contribution statement

Klaudia Adels: Writing – review & editing, Writing – original draft, Validation, Resources, Investigation. **Yulia Monakhova:** Writing – review & editing, Writing – original draft, Methodology, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.microc.2024.110859.

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