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Solvent Free Rapid Analysis of Flavor Compounds in E-Liquid by Stir Bar Sorptive Extraction and Thermal Desorption GC/MS

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Keywords

E-liquid, Flavor, Flavorings, Stir Bar Sorptive Extraction SBSE, Twister, Thermal Desorption

Abstract

With the global surge in the use of electronic cigarettes, there is a corresponding increase in the consumption of e-liquid. However, different regions and countries impose varying regulatory requirements concerning the types and concentrations of flavorings added to electronic liquids (e-liquids). Analyzing the flavorings in e-liquids poses a challenge due to their substantial content of propylene glycol, glycerol solvent, and nicotine as a matrix.

Conventional methods such as traditional solvent extraction and simultaneous distillation/extraction necessitate the use of solvents, leading to time-consuming, labor-intensive, and cumbersome procedures. Additionally, the sensitivity of solid phase microextraction is limited, and headspace analysis may be affected by solvent interference.

In response to these challenges, Stir Bar Sorptive Extraction (SBSE) emerges as a modern, green, and solvent-free sample extraction technology. Renowned for its efficiency, high sensitivity, and user-friendly operation, SBSE proves particularly effective in extracting volatile flavor and aroma components from e-liquids. The analytical process involves utilizing a Cooled Inlet System, thermal desorption, and gas chromatography-mass spectrometry for the analysis and identification of volatile flavor and aroma compounds in e-liquids. The AromaOffice2D flavor compounds database software is then employed to process the GC-MS data, enhancing the precision and efficiency of flavor compound identification.

Introduction

Mandatory National Standard GB 41700-2022

In recent years, the global usage of electronic cigarettes (e-cigarettes) has witnessed a significant increase [1]. This surge has led to the proliferation of numerous e-cigarette companies dedicated to developing and manufacturing e-cigarette refill solutions, commonly known as e-liquids. However, the regulatory landscape for these products varies across different regions and countries, with distinct standards dictating the types and quantities of additives permissible in e-liquids.

Notably, in China, the implementation of the mandatory national standard GB 41700-2022 for electronic cigarettes began on October 1, 2022. This standard explicitly specifies that the characteristic flavor of the product must not exhibit any taste other than tobacco. Furthermore, the regulation states that if electronic cigarettes contain nicotine, they fall under the definition of electronic cigarettes. Consequently, electronic cigarette products without nicotine are prohibited from entering the market for sale quantity. This regulatory framework reflects the evolving landscape of e-cigarette governance and underscores the importance of adherence to specific flavor and nicotine content standards in the Chinese market.

GERSTEL AppNote 268

The national standard GB41700-2022, titled "Electronic Cigarette," outlines specific regulations governing allowable additives and their maximum usage amounts for aerosols. This standard serves as a whitelist, enumerating 101 types of aerosol additives permitted for inclusion in electronic cigarettes. Notably, numerous flavorings that were previously permissible are now prohibited, necessitating research and development personnel to create new formulas that align with the updated regulatory requirements.

It's important to highlight that there are distinct regulations for both the types and dosages of additives in E-liquids destined for export versus those sold domestically. Analyzing the variety and concentrations of flavorings in E-liquids holds significant importance for assessing compliance with corresponding regulations, ensuring the rational addition of flavorings, and studying the market's developmental trends in flavorings used in E-liquids. This analytical approach not only aids in confirming regulatory adherence but also facilitates the formulation of E-liquids that meet evolving standards, ensuring both domestic and international market compatibility for electronic cigarette products.

The Challenge of Analyzing E-liquid

E-liquids are commonly composed of a glycerol (VG) and propylene glycol (PG) base, flavorings, nicotine, and other chemicals. These components undergo heating, aerosolization, and inhalation. The principal constituents of E-liquids, PG and VG, constitute a high concentration ranging from 80-90%. Additionally, the composition includes water (about 2%), nicotine, and a small quantity of flavorings.

Analyzing e-liquids poses unique challenges due to the relatively small content of flavorings. Direct injection methods are limited, as they can only detect larger components and may overlook trace flavoring constituents. Additionally, propylene glycol (PG) and glycerol (VG), the primary components of e-liquids, may co-elute with other flavorings, masking or affecting the determination of these components.

The composition of e-liquids is diverse, typically including 20-50% propylene glycol, 20-50% glycerol, 2% nicotine, and flavorings. The concentration range varies widely, spanning from tens of percent to mg/kg and µg/Kg. The flavorings themselves are a complex mixture, encompassing various forms such as liquid, solid, semi-solid, monomer, essential oil, extract, concrete, and tincture. This diverse array of raw materials results in a mixture rich in volatile and semi-volatile compounds, comprising a wide range of both natural and artificial flavoring materials.

These flavoring compounds consist of numerous chemical classes, including aldehydes, ketones, acids, alcohols, esters, terpenes, sulfur compounds, nitrogen compounds, and others. The chemical properties and structures of these compounds are highly intricate, encompassing polar, non-polar, and heterocyclic characteristics. The extraction, separation, and identification of these compounds are challenging due to their complexity and diversity.

Complicating matters further, there are interactions among these compounds, adding to the difficulty of their identification. Some of these compounds have very low detection thresholds, meaning that even trace amounts can impart a pronounced aroma (e.g., some sulfur and nitrogen compounds).

The intricate nature of the flavoring components in e-liquids requires sophisticated analytical techniques to achieve accurate and comprehensive analysis. Efforts to mitigate the influence of propylene glycol (PG) and glycerol (VG) on the determination of flavoring components, along with reducing interference, are pivotal for achieving precise and reliable analytical results in the study of e-liquids.

Extraction Technique

Traditional methods like solvent extraction (SE), liquid-liquid extraction (LLE), and simultaneous distillation/extraction (SDE) are commonly employed to determine flavoring compounds in e-liquids. However, these methods often demand a substantial amount of solvents, increased sample quantities, and additional concentration steps. Moreover, the potential introduction of side reactions makes these approaches time-consuming, labor-intensive, and cumbersome. Solid phase microextraction has low sensitivity and a small linear range for determination. The headspace will be disturbed by PG and VG solvents.

The adoption of modern techniques like Stir Bar Sorptive Extraction (SBSE) stands as a solvent-free, efficient, and highly sensitive alternative, effectively addressing challenges and providing a more accurate analysis of flavor compounds in e-liquids. This modern, green sample extraction technology proves efficient, highly sensitive, and user-friendly for extracting aroma and flavor components [4]. In the current study, SBSE is employed to extract volatile flavor and aroma components from E-liquids.

GERSTEL AppNote 268

Experimental

Samples

E-liquids designed for use as e-cigarette refill solutions were obtained online from a vendor based in China.

Instrumentation

GERSTEL LabWorks Platform with Cryostatic Cooling Device CCD 2 option combined with Agilent 7890/5975 GC/MSD.

Analysis Conditions LabWorks Platform

SBSE	PDMS twister stir bar
	10 mm length, 1 mm thickness
TDU	Splitless
	25 °C (0.2 min); 100 °C/min; 250 °C (8 min)
CIS	Tenax liner
	Solvent Vent (50 mL/min), split 11:1
	-30 °C (0.5 min); 12 °C/sec; 250 °C (10 min)

Analysis Conditions Agilent GC 7890

Column	60 m HP-Innowax column (Agilent, USA)
	d_i=0.25 mm d_f=0.25 µm
Pneumatics	He (> 99.999%)
	P_i=208.18 kPa, Constant Flow 1.8 mL/min
Oven	40°C (2 min); 5°C/min; 250°C (24 min)
	(note: MSD/ODP split ratio is with 1:1)

Analysis Conditions Agilent 5975 MSD

EI	70 eV
Interface	250 °C
Ion source	230 °C
Quadrupole	150 °C
Full scan	33 – 400 amu

Sample Preparation

The volatile flavor compounds in e-liquid were extracted using the Stir Bar Sorptive Extraction (SBSE) technique using a PDMS Twister stir bar. For each sample, 50 mg (the sample amount depends on the flavoring content) of E-liquid was used. 200 mg/kg 2-nonanol as an internal standard was accurately added to the sample for semi-quantification and placed in a 20 mL headspace vial together with 2 g of saturated NaCl water solution. The PDMS Twister was immersed in the solution and the volatile compounds were extracted for 60 minutes at room temperature on a stirrer with a stirring speed of 1000 rpm. The twister was then removed with tweezers or an assembly tool for TDU, rinsed briefly in distilled water and dried with a clean, lint-free cloth, and then transferred to a thermal desorption tube for subsequent GC-MS analysis.

Thermal Desorption

For the subsequent GC–MS analysis, the PDMS twister was introduced into the Thermal Desorption Unit (TDU 2), adhering to the parameters outlined in the analysis conditions above. Please refer to the specified TDU 2 and CIS 4 parameters for further details.

Data Processing Software

MS ChemStation Data Analysis version F (Agilent Technologies), AromaOffice2D (Gerstel K.K.).

Results and Discussion

Effect of High Content of Propylene Glycol and Glycerol in E-liquids on the Determination of Trace Flavorings

Given that the primary components in the E-liquid are propylene glycol (PG) and vegetable glycerin (VG), the concentration of flavorings is relatively minimal. Direct liquid injection is limited in its ability to detect only larger components, making it inadequate for identifying trace flavoring constituents. Figures 1 and 2 display the Total Ion Chromatogram obtained through GC-MS analysis using direct liquid injection.

GERSTEL AppNote 268

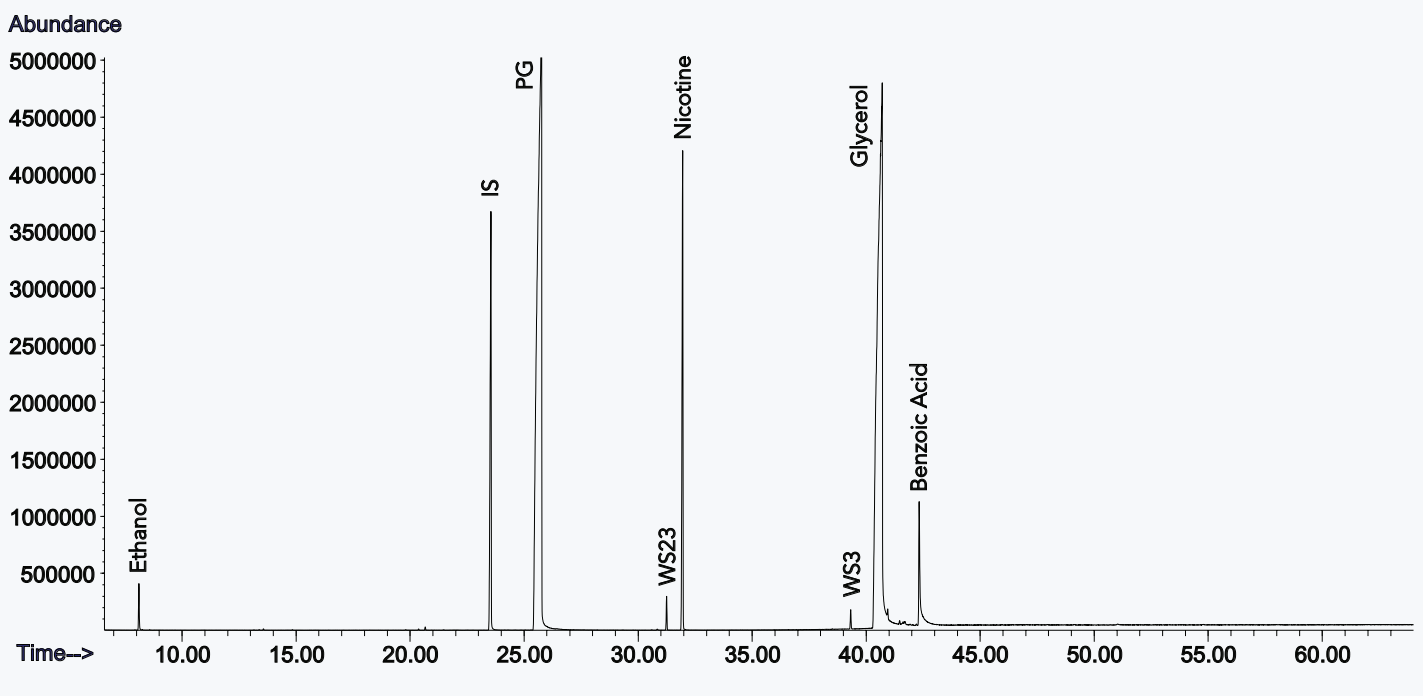

Figure 1: GC-MS total ion chromatogram by direct liquid injection of sample A.

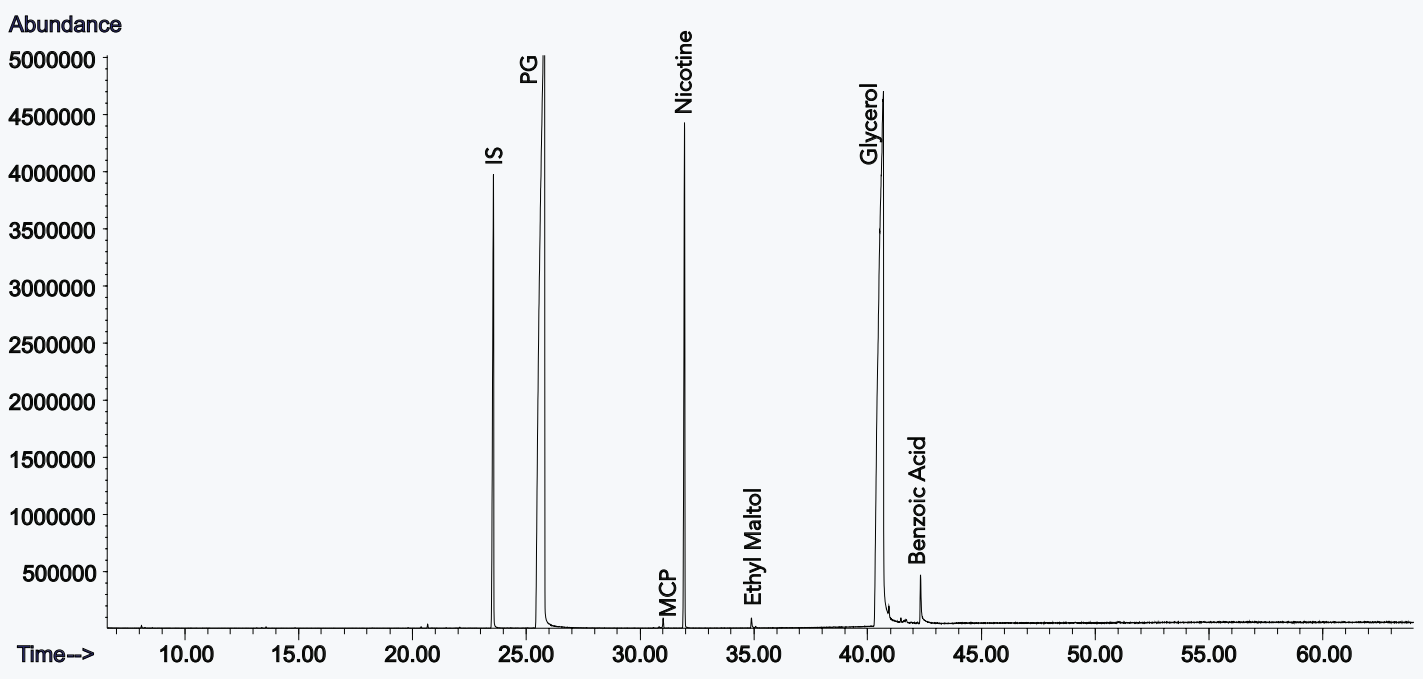

Figure 2: GC-MS total ion chromatogram by direct liquid injection of sample B.

Figures 1 and 2 illustrate that direct liquid injection is primarily effective in identifying solvents and high-content components in E-liquids. Notable components detected include Ethanol, Propylene Glycol (PG), Cooling Agent WS23, Nicotine, Cooling Agent WS3, Glycerol (VG), Benzoic acid, 3-methyl cyclopentenolone-2.2 (MCP), and Ethyl Maltol. However, it is evident that most flavorings present in the E-liquid are not detected through this method.

Indeed, while increasing the injection volume may enhance chromatographic peak intensity, it's crucial to avoid excessively large direct injection volumes. Oversized injection volumes can lead to overloading of propylene glycol (PG) and glycerol (VG). Moreover, there's a risk of co-elution of PG and VG with other flavoring components, potentially masking or influencing the determination of those components. Therefore, it becomes imperative to eliminate the influence of PG and VG on the determination of flavoring components and reduce potential interference. Balancing injection volume is key to achieving accurate and reliable results in the analysis of E-liquids.

Obviously, to determine trace flavorings compounds in E-liquids, it is not enough to directly inject the liquid sample, and it is impossible to obtain complete trace flavorings information. More effective sample preparation methods are necessary to extract trace components from E-liquids.

Figure 3 presents a comparison of Total Ion Chromatogram (TIC) between direct liquid injection and Stir Bar Sorptive Extraction (SBSE) of e-liquid. (sample A).

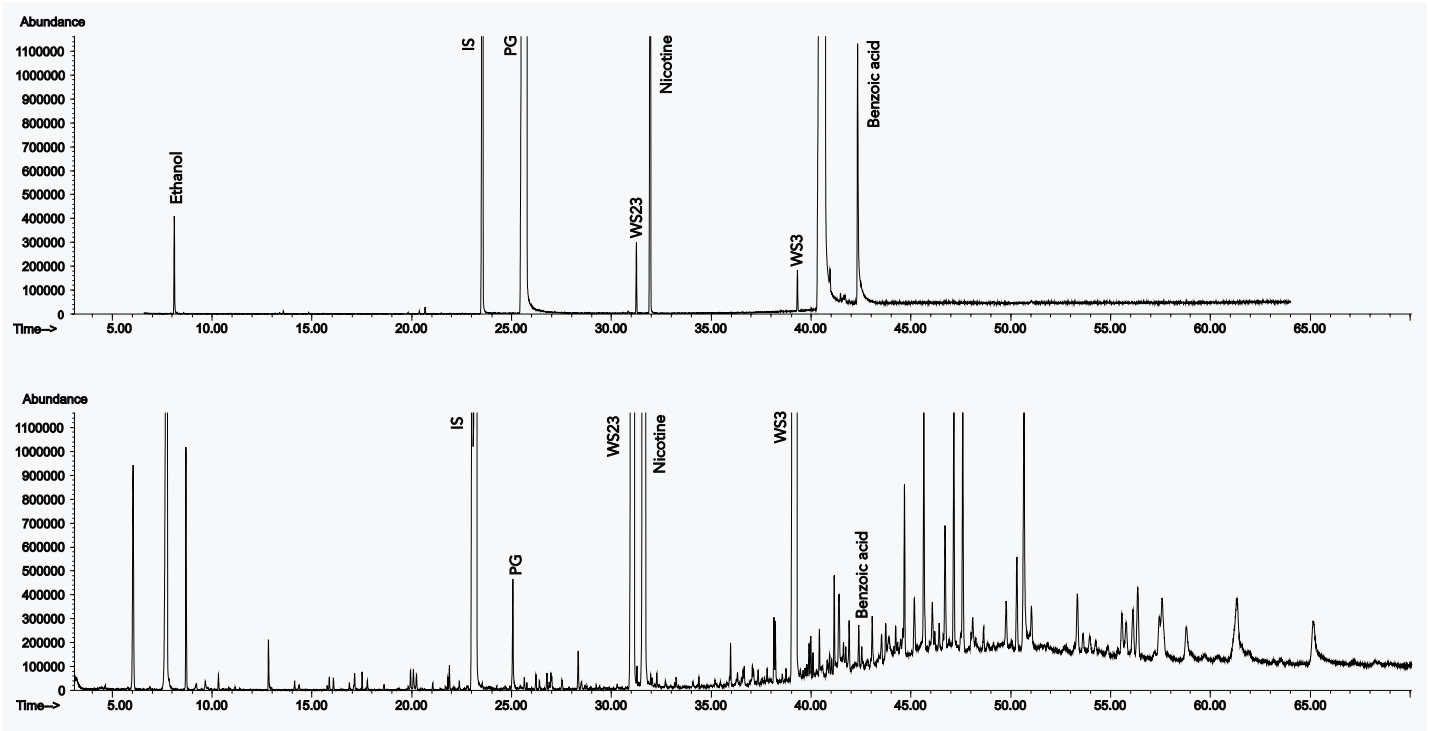

Figure 3: Comparison of TIC between direct liquid injection and SBSE extraction of electronic-liquid (Sample A).

Figure 3 reveals that through direct liquid injection, sample A primarily detected solvents and high-content components in the E-liquids. However, Stir Bar Sorptive Extraction (SBSE) exhibited the capability to detect trace flavoring components. Notably, the peaks of propylene glycol and glycerol solvents are minimal, and the ethanol peak is amplified, likely due to the use of ethanol in the internal standard preparation. A similar pattern is observed for sample B in Figure 4. This emphasizes the enhanced ability of SBSE to capture a broader spectrum of flavoring compounds compared to direct liquid injection.

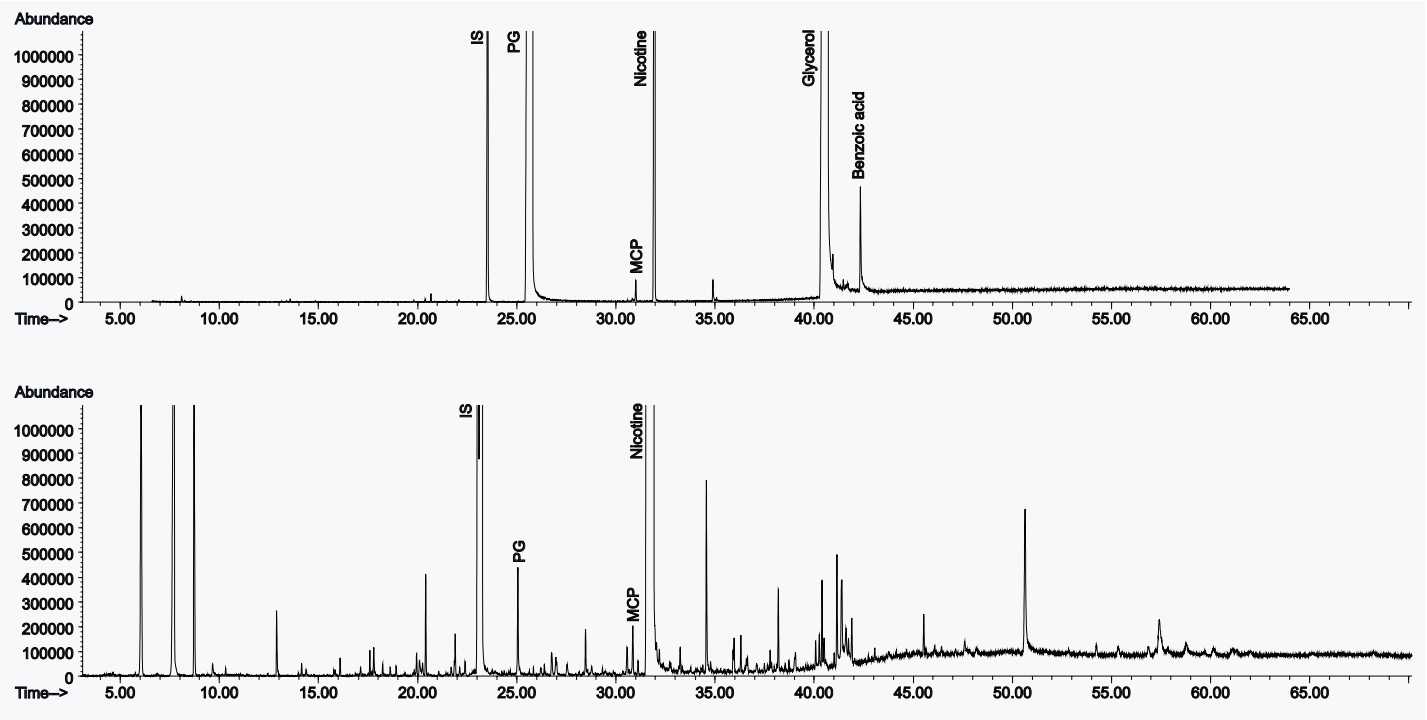

Figure 4: Comparison of TIC between direct liquid injection and SBSE extraction of electronic-liquid (Sample B).

Analysis of Flavorings Compounds in E-liquids

The semi-quantitative internal standard method was used for calculating content of each compound in E-liquids.

Approximately 87 volatile flavor compounds were identified through the extraction of volatile compounds from E-liquid (sample A) using Stir Bar Sorptive Extraction (SBSE), as detailed in Table 1. Notably, the E-liquid exhibits a high concentration of flavorings, with prominent compounds including the cooling agent WS23 (328.8 mg/kg, No.45), nicotine (299.4 mg/kg, No.46) and its derivatives, along with the cooling agent WS3 (451.5 mg/kg, No.64).

Additionally, there are traces of various other flavorings, typically ranging from 0.03-7 mg/kg. Among these are nitrogen compounds and 8 pyridine derivatives, such as pyridine, dimethyl pyridine, and trimethyl pyridine, which collectively contribute to an approximate concentration of 1.712 mg/kg. Furthermore, there are detectable levels of furfural, furfuryl alcohol, ketone compounds, Tabanone (four isomers of approximately 4.898 mg/kg), is from tobacco extracts and are a very important odor compound in tobacco. Dihydroxyactinidinide is detected at 0.513 mg/kg, alongside other aldehydes, ketones, alcohols, acids, esters, phenols, and various compounds.

The polyol structure of propylene glycol (PG) and vegetable glycerin (VG) makes them susceptible to reactions with aldehydes, ketones, acids, and other compounds in e-liquids, leading to the formation of new compounds.

GERSTEL AppNote 268

Table 1: Components of E-liquid (A) by SBSE.

No	RT	RI	Area	mg/kg	RI_Lib	CAS No	Formula	Name (Library)	Name (Aroma-DB)/Remark
1	4.629	624	403197	0.118	652	75-07-0	$C_2H_4O_2$	Acetaldehyde	Acetaldehyde
2	5.77	811	325163	0.095	818	67-64-1	C_3H_6O	Acetone	Acetone
3	6.902	891	161576	0.047	890	105-57-7	$C_6H_{14}O_2$	1,1-Diethoxyethane	1,1-Diethoxyethane/Acetal
4	7.744	934	149369844	43.557	933	64-17-5	C_2H_6O	Ethanol	Ethanol (IS solvent)
5	9.202	1003	992118	0.289		565-80-0	$C_7H_{14}O$	Pentanone, 2,4-dimethyl-3-	
6	10.311	1049	1421416	0.414	1040	108-88-3	C_7H_8	Toluene	Toluene
7	11.146	1083	229148	0.067		15192-80-0	C_8H_{12}	(E,E,E)-2,4,6-Octatriene	
8	11.392	1093	86673	0.025	1083	66-25-1	$C_6H_{12}O$	Hexanal	Hexanal
9	15.872	1249	1111176	0.324		42367-31-7	$C_9H_{18}O$	1-Hexylvinyl methyl ether	
10	16.079	1256	1065123	0.311	1266	108-48-5	C_7H_9N	2,6-Lutidine	2,6-Dimethylpyridine
11	16.468	1270	69359	0.020	1253	100-42-5	C_8H_8	Benzene, ethenyl-	Styrene
12	16.881	1284	593455	0.173		61142-42-5	$C_9H_{18}O$	2-Octene, 2-methoxy-	
13	17.142	1293	1864803	0.544	1281	95-63-6	C_9H_{12}	Benzene, 1,2,4-trimethyl-	Trimethylbenzene
14	17.693	1313	166881	0.049	1298	108-89-4	C_6H_7N	4-Methyl-pyridine	4-Methylpyridine
15	17.88	1320	1057772	0.308		116-09-6	$C_3H_6O_2$	2-oxo-Propanol	
16	18.62	1346	571966	0.167		583-58-4	C_7H_9N	Pyridine, 3,4-dimethyl-	
17	19.285	1370	109401	0.032		583-61-9	C_7H_9N	Pyridine, 2,3-dimethyl-	
18	19.359	1372	208192	0.061	1378	108-75-8	$C_8H_{11}N$	Pyridine, 2,4,6-trimethyl-	2,4,6-Trimethylpyridine
19	19.83	1389	319507	0.093			$C_8H_{11}N$	Pyridine, 2,4,6-trimethyl-, isomer	
20	20.092	1398	2410988	0.703	1392	124-19-6	$C_9H_{18}O$	Nonanal	Nonanal
21	20.53	1416	127283	0.037	1413	123-96-6	$C_8H_{18}O$	2-Octanol	2-Octanol
22	21.061	1437	634488	0.185	1434	106-32-1	$C_{10}H_{20}O_2$	Octanoic acid, ethyl ester	Ethyl octanoate
23	21.435	1452	151285	0.044		696-30-0	$C_8H_{11}N$	4-Isopropylpyridine	
24	21.514	1455	111732	0.033	1457	111-70-6	$C_7H_{16}O$	1-Heptanol	Heptanol
25	21.895	1471	2262592	0.660			$C_9H_{13}N$	Pyridin, 5-isopropyl-2-methyl	
26	22.124	1480	288809	0.084	1463	98-01-1	$C_5H_4O_2$	Furfural	Furfural
27	22.394	1491	975097	0.284	1491	104-76-7	$C_8H_{18}O$	1-Hexanol, 2-ethyl-	2-Ethylhexanol
28	23.241	1525	629624090	183.600	1530	628-99-9	$C_9H_{20}O$	2-Nonanol (IS)	2-Nonanol as IS
29	24.9		321505	0.094		19781-27-2	$C_{10}H_{22}O$	Decan-3-ol	
30	25.059	1599	13308163	3.881		57-55-6	$C_3H_8O_2$	1,2-Propanediol (PG)	PG (solvent)
31	25.632	1623	1039370	0.303		539-88-8	$C_7H_{12}O_3$	Pentanoic acid, 4-oxo-, ethyl ester	Ethyl levulinate
32	26.217	1648	1464585	0.427	1633	1490-04-6	$C_{10}H_{20}O$	Cyclohexanol, 5-methyl-2-(1-methylethyl)-	Menthol
33	26.388	1656	1011007	0.295		533-37-9	C_8H_9N	5H-1-Pyrindine, 6,7-dihydro-	
34	26.74		68744	0.020		98-00-0	$C_5H_6O_2$	Furfuryl alcohol	
35	26.768	1672	1714317	0.500		990056-03-9	$C_{10}H_{22}O_3$	(2-(2-butoxyisopropoxy)-2-propanol	DPG

Table 1 (cont.): Components of E-liquid (A) by SBSE.

No	RT	RI	Area	mg/kg	RI_Lib	CAS No	Formula	Name (Library)	Name (Aroma-DB)/Remark
36	26.967	1681	1575963	0.460		55956-25-7	$C_9H_{18}O_3$	2-Propanol, 1-[1-methyl-2-(2-propenyloxy)ethoxy]-	DPG
37	27.01	1682	953004	0.278				DPG	DPG
38	27.08		11617	0.003				Ethyl benzoate	
39	27.52	1705	1215146	0.354				DPG	DPG
40	28.334	1741	4229794	1.233	1718	1937-54-8	$C_{13}H_{22}O$	Solanone E	
41	28.504	1749	1114103	0.325			$C_9H_{18}O_3$	Propylene glycol trimer 5	
42	28.674	1757	356428	0.104			$C_9H_{18}O_3$	Propylene glycol trimer 6	
43	28.773	1761	304764	0.089		5413-49-0	$C_{10}H_{18}O_4$	1,3-Dioxolane-2-propanoic acid, 2,4-dimethyl-, ethyl ester	Ethyl levulinate PG ketal 1
44	29.41	1790	319110	0.093		10493-98-8	$C_5H_6O_2$	2-Hydroxy-2-cyclopenten-1-one	
45	31.152	1872	1127510834	328.785				Cooling agent W23	
46	31.711	1898	1026841458	299.430		75202-10-7	$C_{10}H_{14}N_2$	Nicotine	
47	31.973	1911	1161011	0.339	1896		$C_9H_{18}O_3$	2-Hydroxypropyl capronate	Acid+PG reaction
48	32.043	1914	950594	0.277				Ethyl levulinate reaction	
49	32.278	1926	1117036	0.326		504-96-1	$C_{20}H_{38}$	Neophytadiene	
50	32.706	1947	1032533	0.301				2-Hydroxypropyl capronate	Acid+PG reaction
51	33.245	1974	1022773	0.298				Nicotine derivative	
52	35.456	2087	575313	0.168		1502-22-3	$C_{12}H_{18}O$	2-(1-Cyclohexenyl)cyclo-hexanone	
53	35.906	2111	800031	0.233		990065-59-1	$C_{10}H_{13}ClN_2$	nicotine relative	
54	35.959	2113	4055121	1.182		68332-79-6	$C_{11}H_{22}O_3$	2-Hydroxypropyl octanoate	Acid+PG reaction
55	36.641	2150	2011797	0.587			$C_{11}H_{22}O_3$	1-Hydroxy-2-propyl octa-noate	PG+ Acid
56	37.048	2172	3061809	0.893		60619-46-7	$C_{12}H_{20}O_2$	Norsoladione	
57	37.103	2175	2056087	0.600	2167	112-05-0	$C_9H_{18}O_2$	Nonanoic acid	Nonanoic acid
58	37.332	2187	1576034	0.460	2193	013215-88-8	$C_{13}H_{18}O$	Megastigmatrien-3-one, 4,6Z,8E-	Tabanone
59	37.653	2205	652382	0.190		29419-55-4	$C_{10}H_{14}N_2O$	Nicotine, 1'-oxide	
60	38.124	2231	6647165	1.938		38818-55-2	$C_{13}H_{18}O$	Megastigmatrienone	Tabanone
61	38.191	2235	6691610	1.951		1125-96-8	$C_9H_{10}N_2$	Myosmine	
62	38.545	2255	910752	0.266				Levulinic acid reaction	
63	38.724	2265	1592253	0.464				Levulinic acid reaction	
64	39.274	2296	1548345825	451.502		39711-79-0	$C_{13}H_{25}NO$	Cyclohexanecarboxam-ide, N-ethyl-5-meth-yl-2-(1-methylethyl)-	Cooling agent WS2
65	39.452	2306	1799986	0.525		13215-88-8	$C_{13}H_{18}O$	Megastigmatrien-3-one, 4,6Z,8Z-	Tabanone

Table 1 (cont.): Components of E-liquid (A) by SBSE.

No	RT	RI	Area	mg/kg	RI_Lib	CAS No	Formula	Name (Library)	Name (Aroma-DB)/Remark
66	39.59	2314	1264226	0.369				Levulinic acid reaction	
67	39.697	2321	1730642	0.505				Phenol, 2,4-di-tert.-butyl-	
68	39.783	2326	1899538	0.554				Megastigmatrienone	Tabanone
69	39.977	2337	4873333	1.421		13215-88-8	$C_{13}H_{18}O$	Megastigmatrien-3-one, 4,6E,8Z-	Tabanone
70	40.089	2343	2197486	0.641				Levulinic acid reaction	
71	40.41	2362	3790740	1.105		487-19-4	$C_{10}H_{10}N_2$	Nicotyrine	
72	41.029	2398	1759464	0.513		990042-72-9	$C_{11}H_{16}O_2$	Dihydroactinidiolide	
73	41.153	2406	9760342	2.846				Levulinic acid reaction	
74	41.401	2421	13793533	4.022				Levulinic acid reaction	
75	41.619	2434	4928939	1.437				Levulinic acid reaction	
76	41.735	2441	3221176	0.939				Levulinic acid reaction	
77	41.903	2452	6844210	1.996				Levulinic acid reaction	
78	43.059	2523	8791563	2.564		581-50-0	$C_{10}H_8N_2$	2,3'-Dipyridyl	
79	43.736	2565	6376022	1.859			$C_{22}H_{26}O_4$	Diisobutyl phthalate	
80	44.593	2618	3953401	1.153				phytol	
81	46.084	2706	8924815	2.602	2704	544-63-8	$C_{14}H_{28}O_2$	Tetradecanoic acid	Myristic acid
82	46.423	2723	3239131	0.945		84-74-2	$C_{16}H_{22}O_4$	Dibutyl phthalate	
83	50.664	2918	68720786	20.039	2909	57-10-3	$C_{16}H_{32}O_2$	n-Hexadecanoic acid	Palmitic acid
84	57.452	3129	10880920	3.173		990264-67-0	$C_{18}H_{36}O_2$	Stearic acid	
85	58.804	3162	12983130	3.786	3178	112-80-1	$C_{18}H_{34}O_2$	Oleic acid	Oleic acid
86	61.338	3219	25082929	7.314		506-21-8	$C_{18}H_{32}O_2$	Linoelaidic acid	
87	65.152	3293	17287036	5.041		463-40-1	$C_{18}H_{30}O_2$	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	Linoleic acid

The content and types of volatile compounds in e-liquid (sample B) differ from those in sample A. Approximately 105 volatile flavor compounds were identified as detailed in Table 2. Nicotine (1147.5 mg/kg, No. 49) and its derivatives exhibit the highest content. Notably, the cooling agent WS23 is relatively low at 0.4615 mg/kg (No.48). Various other flavorings are present in trace amounts, generally ranging from 0.07 to 5.76 mg/kg. For instance, there are approximately 9 pyridines total about 2.537 mg/kg and 4 pyrazines total about 3.422 mg/kg, both higher than in sample A. Additionally, there are some furfural, furfuryl alcohol, ketone compounds, including Beta Damascenone (1.145 mg/kg, No.46), a commonly used flavor compound in tobacco. Tabanone (No.73 and No.82) , is at approximately 0.399 mg/kg, a very important odor compound in tobacco derived from tobacco extracts. 3-methylcyclohexenolone 2.2 (MCP) is at 1.769 mg/kg (No.47), while dihydroactinidiniolide is not found. Ethyl Maltol is about 5.763 mg/kg. Benzyl acetate is at 1.28 mg/kg (No.39), Benzyl benzoate at 1.214 mg/kg, and Benzyl cinnamate at 0.856 mg/kg. Various other compounds include aldehydes, ketones, alcohols, acids, esters, phenols, etc.

Additionally, there are acetals, ketals, and esters generated by reactions of aldehydes, ketones, and acids with PG or VG. Some DPG and PG trimmers are also present. Levulinic acid and PG/VG generate multiple complex ketal and ester compounds at approximately 20.236 mg/kg, exceeding the levels found in sample A. The Levulinic acid itself disappeared.

GERSTEL AppNote 268

Table 2: Components of E-liquid (B) by SBSE.

No	RT	RI	Area	mg/kg	RI_Lib	CAS No	Formula	Name (Library)	Name (Aroma-DB)/Remark
1	4.639	624	530697	0.1468	652	75-07-0	$C_2H_4O_2$	Acetaldehyde	Acetaldehyde
2	5.768	811	500196	0.1384	818	67-64-1	C_3H_6O	Acetone	Acetone
3	6.872	889	380366	0.1052	890	105-57-7	$C_6H_{14}O_2$	1,1-Diethoxyethane	1,1-Diethoxyethane/Acetal
4	7.718	933	101841313	28.171	933	64-17-5	C_2H_6O	Ethanol	Ethanol (IS solvent)
5	10.308	1049	637869	0.1764	1040	108-88-3	C_7H_8	Toluene	Toluene
6	11.379	1093	176170	0.0487	1083	66-25-1	$C_6H_{12}O$	Hexanal	Hexanal
7	12.433	1130	271947	0.0752	1125	123-92-2	$C_7H_{14}O_2$	1-Butanol, 3-methyl-, acetate	Isoamyl acetate
8	14.374	1197	852120	0.2357	1187	110-85-1	C_5H_5N	Pyridine	Pyridine
9	15.83	1247	740786	0.2049		42367-31-7	$C_9H_{18}O$	1-Hexylvinyl methyl ether	
10	16.083	1256	1671933	0.4625	1266	108-48-5	C_7H_9N	2,6-Lutidine	2,6-Dimethylpyridine
11	17.118	1292	1038386	0.2872	1281	95-63-6	C_9H_{12}	Benzene, 1,2,4-trimethyl-	Trimethylbenzene
12	17.229	1296	154897	0.0428	1290	124-13-0	$C_8H_{16}O$	Octanal	Octanal
13	17.506	1306	352925	0.0976	1290	108-99-6	C_6H_7N	Pyridine, 3-methyl-	3-Methylpyridine
14	17.693	1313	298432	0.0826	1298	108-89-4	C_6H_7N	4-Methyl-pyridine	4-methylpyridine
15	17.784	1316	3276425	0.9063	1300	116-09-6	$C_3H_6O_2$	2-Propanone, 1-hydroxy-	Acetol
16	18.234	1332	1075043	0.2974	1327	123-32-0	$C_6H_8N_2$	Pyrazine, 2,5-dimethyl-	2,5-Dimethylpyrazine
17	18.617	1346	841493	0.2328		108-47-4	C_7H_9N	Pyridine, 2,4-dimethyl-	
18	18.912	1356	948352	0.2623	1348	5910-89-4	$C_6H_8N_2$	Pyrazine, 2,3-dimethyl-	2,3-Dimethylpyrazine
19	19.279	1369	130338	0.0361		583-58-4	C_7H_9N	Pyridine, 3,4-dimethyl-	
20	19.362	1372	324528	0.0898	1378	108-75-8	$C_8H_{11}N$	Pyridine, 2,4,6-trimethyl-	2,4,6-trimethylpyridine
21	19.766	1387	283245	0.0783		5077-67-8	$C_4H_8O_2$	1-Hydroxy-2-butanone	
22	19.829	1389	565241	0.1564		1462-84-6	$C_8H_{11}N$	Pyridine, 2,3,6-trimethyl-	
23	20.094	1399	2854793	0.7897	1392	124-19-6	$C_9H_{18}O$	Nonanal	Nonanal
24	20.406	1411	9224989	2.5518	1405	14667-55-1	$C_7H_{10}N_2$	2,3,5-Trimethylpyrazine	Trimethylpyrazine
25	21.063	1437	473805	0.1311	1434	106-32-1	$C_{10}H_{20}O_2$	Octanoic acid, ethyl ester	Ethyl octanoate
26	21.688	1462	1042852	0.2885	1451	64-19-7	$C_2H_4O_2$	Acetic acid	Acetic acid
27	21.893	1471	4135181	1.1439			$C_9H_{13}N$	5-Isopropyl-2-methyl-pyridine	
28	22.12	1480	1053943	0.2915	1463	98-01-1	$C_5H_4O_2$	Furfural	Furfural
29	22.395	1491	1879006	0.5198	1491	104-76-7	$C_8H_{18}O$	1-Hexanol, 2-ethyl-	2-Ethylhexanol
30	23.253	1525	739655222	204.6	1530	628-99-9	$C_9H_{20}O$	2-Nonanol	2-Nonanol/IS
31	25.052	1599	11523532	3.1876		57-55-6	$C_3H_8O_2$	1,2-Propanediol	PG (solvent)
32	25.63	1623	539393	0.1492		539-88-8	$C_7H_{12}O_3$	Levulinic acid, ethyl ester	
33	26.212	1648	1123884	0.3109	1633	1490-04-6	$C_{10}H_{20}O$	Cyclohexanol, 5-methyl-2-(1-methylethyl)-	Menthol/eluted 2-Acetyl pyrazine
34	26.386	1656	912026	0.2523		533-37-9	C_8H_9N	5H-1-Pyrindine, 6,7-dihy-dro-	
35	26.755	1672	3510293	0.971	1661	98-00-0	$C_5H_6O_2$	Furfuryl alcohol	Furanmethanol
36	26.965	1681	2325976	0.6434				DPG	
37	27.009	1682	1191346	0.3295				DPG	
38	27.534	1705	2070637	0.5728			$C_9H_{18}O_3$	Propylenglycol trimer	PG trimer

Table 2 (cont.): Components of E-liquid (B) by SBSE.

No	RT	RI	Area	mg/kg	RI_Lib	CAS No	Formula	Name (Library)	Name (Aroma-DB)/Remark
39	28.465	1747	4640389	1.2836	1740	140-11-4	$C_9H_{10}O_2$	Acetic acid, phenylmethyl ester	Benzyl acetate
40	28.562	1752	770527	0.2131				PG trimer	PG trimer
41	28.775	1761	1516432	0.4195		5413-49-0	$C_{10}H_{18}O_4$	1,3-Dioxolane-2-propanoic acid, 2,4-dimethyl-, ethyl ester	Ethyl levulinate PG Ketal1
42	29.483	1793	882982	0.2442		10493-98-8	$C_5H_6O_2$	2-Hydroxy-2-cyclopent-en-1-one	
43	30.364	1834	742195	0.2053			$C_{13}H_{20}O$	Isodamascone	
44	30.364	1834	742195	0.2053	1800	98-85-1	$C_8H_{10}O$	Benzenemethanol, .alpha.-methyl-	1-Phenylethanol
45	30.562	1844	4141607	1.1456	1832	35044-68-9	$C_{13}H_{20}O$	2-Buten-1-one, 1-(2,6,6-trimethyl-1-cyclo-hexen-1-yl)-	β-Damascone
46	30.658	1848	887057	0.2454	1830	23696-85-7	$C_{13}H_{18}O$	Damascenone, beta-	Damascenone
47	30.85	1857	6395611	1.7691	1839	80-71-7	$C_6H_8O_2$	Methyl cyclopentenolone, 3,2,2-	Cyclotene (MCP)
48	31.114	1870	1681477	0.4651		51115-67-4	$C_{10}H_{21}NO$	Cooling agent WS 23	Cooling agent WS 23
49	31.902	1902	4148256339	1147.5		75202-10-7	$C_{10}H_{14}N_2$	Nicotine	eluted 2-Hydroxypropyl capronate
50	32.044	1914	996564	0.2757		20279-49-6	$C_{10}H_{18}O_3$	Pentanoic acid, 4-oxo-, pentyl ester	Levulinic acid reaction
51	32.189	1922	1295087	0.3582		990038-16-3	$C_{12}H_{16}O$	Nicotine derivative	
52	32.705	1947	673867	0.1864				1-Hydroxypropyl capronate	Acid+PG reaction
53	33.238	1973	2711352	0.75				Nicotine derivative	
54	33.342	1979	700415	0.1937				Nicotine derivative	
55	33.772	2000	670173	0.1854				Nicotine derivative	
56	34.013	2012	927614	0.2566		19730-04-2	$C_{11}H_{16}N_2$	1-Methyl-2-(3-pyridinyl) piperidine	
57	34.117	2018	460638	0.1274				Levulinic acid reaction	Levulinic acid reaction
58	34.291	2027	620032	0.1715	2006	108-95-2	C_6H_6O	Phenol & Levelulinic acid reaction	Phenol
59	34.378	2031	893868	0.2473				Nicotine derivative	
60	34.56	2041	20834190	5.7631			$C_7H_8O_3$	Ethyl maltol = y-Pyron, 2-ethyl-3-hydroxy-	
61	34.77	2051	1084106	0.2999	2044	3658-77-3	$C_6H_8O_3$	2,5-Dimethyl-4-hy-droxy-3(2H)-furanone	Furaneol
62	35.097	2068	284766	0.0788	2064	124-07-2	$C_8H_{16}O_2$	Octanoic acid	Caprylic acid
63	35.613	2095	327019	0.0905		95-65-8	$C_8H_{10}O$	Phenol, 3,4-dimethyl-	
64	35.903	2110	2081145	0.5757				Nicotine derivative	
65	35.954	2113	3328720	0.9208		68332-79-6	$C_{11}H_{22}O_3$	2-Hydroxypropyl octanoate	Acid+PG reaction

GERSTEL AppNote 268

Table 2 (cont.): Components of E-liquid (B) by SBSE.

No	RT	RI	Area	mg/kg	RI_Lib	CAS No	Formula	Name (Library)	Name (Aroma-DB)/Remark
66	36.579	2147	1594231	0.441				Levulinic acid reaction	
67	36.634	2150	1447824	0.4005				1-Hydroxypropyl octanoate	
68	37.093	2175	1714145	0.4742				Nonanoic acid & unknown	
69	37.484	2196	883916	0.2445	2191	123-07-9	$C_8H_{10}O$	Phenol, 4-ethyl-	4-Ethylphenol
70	37.484	2196	883916	0.2445	2194	620-17-7	$C_8H_{10}O$	Phenol, 3-ethyl-	3-Ethylphenol
71	37.643	2204	1163012	0.3217				Nicotine oxide	
72	37.843	2216	1230342	0.3403				Nicotine derivative	
73	38.115	2231	882908	0.2442		38818-55-2	$C_{13}H_{18}O$	Megastigmatrienone	Tabanone
74	38.187	2235	8766623	2.425		1125-96-8	$C_9H_{10}N_2$	Myosmine	
75	38.538	2255	1311668	0.3628				Levulinic acid reaction	
76	38.724	2265	1114763	0.3084				Levulinic acid reaction	
77	39.008	2281	1340404	0.3708				Cooling agent WS3	Cooling agent WS4
78	39.441	2306	323934	0.0896		132-64-9	$C_{12}H_8O$	Dibenzofuran	
79	39.566	2313	578340	0.16				Levulinic acid reaction	
80	39.679	2319	369312	0.1022	2321	96-76-4	$C_{14}H_{22}O$	2,4-Di-tert-butylphenol	2,4-Ditertbutylphenol
81	39.844	2329	423411	0.1171		131-11-3	$C_{10}H_{10}O_4$	Dimethyl phthalate	
82	39.948	2335	558867	0.1546		38818-55-2	$C_{13}H_{18}O$	Megastigmatrienone	Tabanone
83	40.071	2342	2931255	0.8108				Levulinic acid reaction	
84	40.254	2353	3875376	1.072				Levulinic acid reaction	
85	40.392	2361	8210994	2.2713		487-19-4	$C_{10}H_{10}N_2$	Nicotyrine	
86	40.495	2367	3191716	0.8829				Levulinic acid reaction	
87	41.005	2397	1550643	0.4289		84-66-2	$C_{12}H_{14}O_4$	Diethyl phthalate	
88	41.144	2405	14145828	3.913				Levulinic acid reaction	
89	41.39	2420	20057631	5.5482				Levulinic acid reaction	
90	41.605	2433	9222827	2.5512				Levulinic acid reaction	
91	41.725	2441	3127579	0.8651				Levulinic acid reaction	
92	41.892	2451	5430502	1.5022				Levulinic acid reaction	
93	42.727	2502	820719	0.227		6766-82-1	$C_{11}H_{16}O_3$	Phenol, 2,6-dimethoxy-4-propyl-	
94	43.053	2522	1137996	0.3148		581-50-0	$C_{10}H_8N_2$	2,3'-Dipyridyl	
95	45.524	2673	4389981	1.2143	2640	120-51-4	$C_{14}H_{12}O_2$	Benzyl benzoate	Benzyl benzoate
96	46.066	2705	987089	0.273	2704	544-63-8	$C_{14}H_{28}O_2$	Tetradecanoic acid	Myristic acid
97	46.419	2723	1119700	0.3097		84-74-2	$C_{16}H_{22}O_4$	Phthalsaeuredibutylester	
98	47.588	2784	1695334	0.469		120-12-7	$C_{14}H_{10}$	Anthracene	
99	50.631	2917	28700251	7.9389	2909	57-10-3	$C_{16}H_{32}O_2$	n-Hexadecanoic acid	Palmitic acid
100	55.33	3073	2205145	0.61		7683-64-9	$C_{30}H_{50}$	Supraene	
101	56.861	3115	2239060	0.6194				Levulinic acid reaction	

Table 2 (cont.): Components of E-liquid (B) by SBSE.

No	RT	RI	Area	mg/kg	RI_Lib	CAS No	Formula	Name (Library)	Name (Aroma-DB)/Remark
102	57.221	3124	1219431	0.3373				Levulinic acid reaction	
103	57.411	3128	12539861	3.4687		990264-67-0	$C_{18}H_{36}O_2$	Stearic acid	
104	58.764	3161	2959439	0.8186	3178	112-80-1	$C_{18}H_{34}O_2$	Oleic acid	Oleic acid
105	60.13	3194	3097163	0.8567		103-41-3	$C_{16}H_{14}O_2$	Benzyl cinnamate	

Conclusion

Stir bar sorptive extraction (SBSE) represents a contemporary, environmentally friendly, and solvent-free sample extraction technology. It is known for its efficiency, high sensitivity, and ease of operation in extracting aroma and flavor compounds from E-liquids. In the case of E-liquid (sample A), approximately 87 volatile flavor compounds were identified using SBSE. Furthermore, E-liquid (sample B) revealed the identification of approximately 105 volatile flavor compounds through the same SBSE technique.

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