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Application News

High Performance Liquid Chromatograph LC-2050C

Analysis of Eight Carbonyl Compounds in E-Cigarette Liquid using High-Performance Liquid Chromatography

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User Benefits

- Enabled simultaneous analysis of eight carbonyl compounds in E-Cigarette Liquid.
- This method can be applied for monitoring the risks of e-cigarettes.

■ Introduction

An e-cigarette is an electronic product that simulates a cigarette. It has the same look, smoke, taste, and feel as a cigarette. It uses a rechargeable lithium polymer battery to power an atomizer that heats e-liquid in the chamber/tank to turn nicotine and other substances into a vapor for the user to inhale. Over the last few years, e-cigarettes have become a new option for many smokers as a substitute for cigarettes, and they are highly sought after as a "healthy" smoking cessation tool. However, e-cigarettes release carbonyl compounds such as formaldehyde, acetaldehyde, and other harmful substances during the heating process, and they can also change the composition of certain chemicals, resulting in new potential hazards. The E-Liquid Safety and Technical Specifications (Draft for Comment) are organizational standards of the China Electronics Chamber of Commerce. Appendix C (E-liquids: Testing for carbonyl compounds) of those specifications stipulates that limit value of formaldehyde, acetone, acrolein, propionaldehyde, crotonaldehyde, 2-butanone, and butyraldehyde in e-cigarette liquid must be ≤20 mg/kg while acetaldehyde must be ≤180 mg/kg.

In this experiment, a method was developed based on Appendix C (E-liquids: Testing for carbonyl compounds) to analyze eight carbonyl compounds in e-cigarette liquids by using high-performance liquid chromatography.

■ Experiment

System

The Sample was analyzed Using LC-2050C 3D, shimadzu high performance liquid chromatograph, and chromatography workstation LabSolutions Ver. 5.98.

Analytical Conditions

Analytical Conditions are shown in Tabel 1.

Table 1 Analytical Conditions

Column	Shim-pack™ GIST C18-HP (150 mm × 4.6 mm I.D., 5 µm)*1
Mobile Phase A	Water/acetonitrile/tetrahydrofuran/isopropyl alcohol = 63:27:9:1
Mobile Phase B	Water/acetonitrile/tetrahydrofuran/isopropyl alcohol = 40:58:1:1
Mobile Phase C	Acetonitrile
Flow rate	1.25 mL/min
Column Temp.	50 °C
Wavelength	UV at 365 nm
Elution mode	Gradient elution, time program is shown in Table 2.

*1 P/N: 227-30041-05

Table 2 Gradient Time Program

Time (min)	Mobile Phase A (%)	Mobile Phase B (%)	Mobile Phase C (%)
0.00	95	0	5
1.00	95	0	5
16.00	70	30	0
19.00	40	60	0
27.00	40	60	0
33.00	0	100	0
35.00	0	0	100
37.00	0	0	100
37.01	95	0	5
41.00	95	0	5

Sample Preparation

- **Mix standard reference solution:** Based on the Draft for Comment, properly transfer DNPH(2,4-Dinitrophenylhydrazine)-derivatized standard solutions of the eight carbonyl compounds to prepare standard stock solutions at a concentration of 10 mg/L. Properly transfer each standard stock solution to prepare a series of standard working solutions with a concentration of 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, and 5.0 mg/L. Prepare at the time of use.
- **The derivatization reagent:** Weigh 4.5 g of DNPH dissolved in about 200 mL of acetonitrile, add 20 mL of 10 % phosphoric acid in aqueous solution, transfer to a 500-mL volumetric flask, add about 250 mL of water, and adjust to volume with acetonitrile. The prepared solution should be stored in a brown reagent bottle away from sunlight, and it can be stored for up to six months.
- **sample solutions:** Accurately weigh 0.1 g of e-cigarette liquid in a brown 1-mL volumetric flask to 0.1 mg, add about 0.8 mL of derivatization reagent, shake well, leave to stand at room temperature for 20 minutes, add 50 µL of pyridine, dilute with acetonitrile, and adjust the volume to reach the mark. Filter through a PTFE membrane and place in a chromatography vial for analysis.

Fig. 1 Appearance of LC-2050C 3D

■ Results and discussion

Chromatogram:

The standard working mixtures (2.0 mg/L) were tested in accordance with the conditions described in Table 2. The chromatogram is shown in Fig. 2. Characteristic peaks of DNPH derivatives of the eight carbonyl compounds were confirmed. Peak resolution of DNPH derivatives of acetone and acrolein was 1.636, which met the requirements.

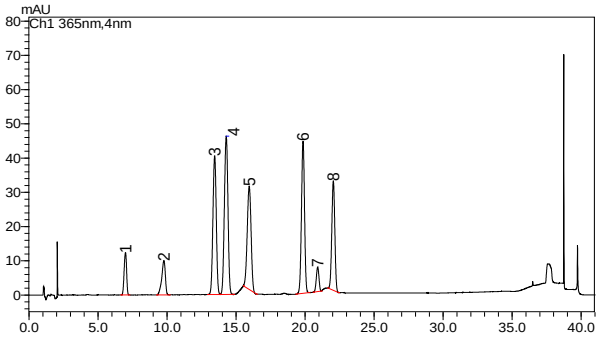

Fig. 2 Chromatogram for Mix standard reference solution (2.0 mg/L)
1. Formaldehyde, 2. Acetaldehyde, 3. Acetone, 4. Acrolein, 5. Propionaldehyde, 6. Crotonaldehyde, 7. 2-butanone, 8. Butyraldehyde

Linearity:

Calibration curves for eight carbonyl compounds were calculated from analysis data of standard working solutions. A good linear correlations were acquired in the concentration range of 0.05 ~ 5.0 mg/L. These results are shown in Fig. 3. Correlation coefficients were all greater than 0.999. Specific results are shown in Table 3.

Table 3 Calibration Curve Parameters

No.	Compound	Linear Equation	Correlation coefficient	Accuracy (%)
1	Formaldehyde	Y = 76218.3X +162.744	0.9997	85.6-116.4
2	Acetaldehyde	Y = 87274.6X -949.451	0.9996	92.8-117.4
3	Acetone	Y = 333463X -2749.83	0.9999	93.5-110.9
4	Acrolein	Y =406256X -2084.23	0.9998	92.1-113.7
5	Propionaldehyde	Y = 338068X -5562.75	0.9998	92.9-112.3
6	Crotonaldehyde	Y = 327883X -3356.25	0.9999	92.2-110.8
7	2-butanone	Y = 39911.6X -547.297	0.9997	86.3-109.8
8	Butyraldehyde	Y = 206013X -1614.52	0.9998	93.2-106.8

Sensitivity:

Sensitivity was calculated at 0.05 mg/L, the lowest concentration of the standard working solutions (the method's detection limit: 0.5 mg/kg). Limit of detection (LOD) and limit of quantitation (LOQ) were calculated using the LabSolutions software and results are shown in Table 4.

Table 4 Sensitivity with Respect to the Eight Carbonyl Compounds

No.	Compound	LOD (mg/kg)	LOQ (mg/kg)
1	Formaldehyde	0.023	0.077
2	Acetaldehyde	0.036	0.120
3	Acetone	0.009	0.030
4	Acrolein	0.008	0.027
5	Propionaldehyde	0.013	0.045
6	Crotonaldehyde	0.010	0.033
7	2-butanone	0.063	0.210
8	Butyraldehyde	0.013	0.044

Recovery:

A standard stock solution was precisely added to a sample of e-cigarette liquid (spike: 20.0 mg/kg), and the sample was prepared like the sample solutions prepared. Recovery results are shown in Table 5. Results revealed that recovery rates of the eight carbonyl compounds were 87.4 to 99.9 %, which met the requirements.

Table 5 Recovery Results (n = 4)

No.	Compound	Volume Added (mg/kg)	Recovery Rate %
1	Formaldehyde		99.9
2	Acetaldehyde		98.4
3	Acetone		97.3
4	Acrolein	20.0	97.2
5	Propionaldehyde		96.0
6	Crotonaldehyde		96.3
7	2-butanone		89.7
8	Butyraldehyde		87.4

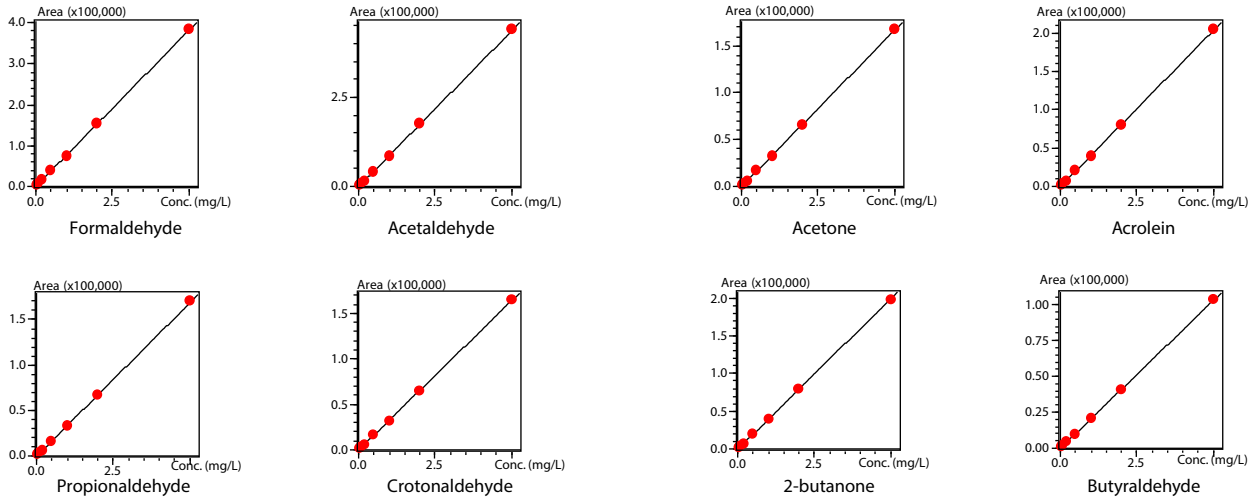

Fig. 3 Calibration Curves for Eight Carbonyl Compounds

Precision:

Standard working solutions (0.1 mg/L, 0.5 mg/L, and 2.0 mg/L) were injected six times consecutively in order to examine instrument precision. Results are shown in Table 6. The retention time RSD% for the eight carbonyl compounds was 0.03 to 0.22 % and the peak area RSD% was 0.10 to 4.29 %. Instrument precision was good.

Testing of e-cigarette liquids:

Six samples of e-cigarette liquids prepared were analyzed in accordance with analytical conditions described in Table 2. The chromatogram for the sample solutions is shown in Fig. 4 and test results are shown in Table 7.

The E-Liquid Safety and Technical Specifications (Draft for Comment) are organizational standards of the China Electronics Chamber of Commerce. Pursuant to Appendix C (E-liquids: Testing for carbonyl compounds) of those specifications, a method is required to meet a detection limit of 0.5 mg/kg for each carbonyl compound. The regulated values for formaldehyde, acetone, acrolein, propionaldehyde, crotonaldehyde, 2-butanone, and butyraldehyde are ≤20 mg/kg while its regulated one for acetaldehyde is ≤180 mg/kg.

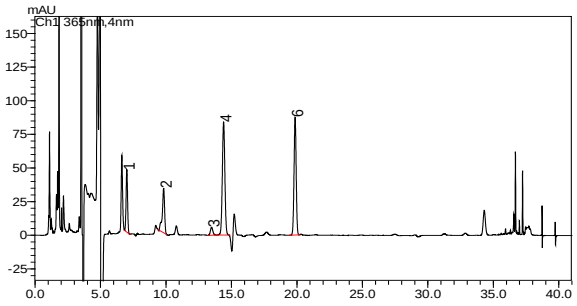

Fig. 4 Chromatogram for E-Cigarette Liquid Sample Solutions (grapefruit, mango, and coconut milk)
1. Formaldehyde, 2. Acetaldehyde, 3. Acetone, 4. Acrolein, 6. Crotonaldehyde

■ Conclusion

In this experiment, a method of using high-performance liquid chromatography to analyze eight carbonyl compounds in e-cigarette liquids was devised based on Appendix C (E-liquids: Testing for carbonyl compounds) of the E-Liquid Safety and Technical Specifications (Draft for Comment). The excellent linearity, repeatability and recovery were confirmed. Additionally, this method was highly sensitive and accurate, so it can provide the tobacco industry with a reference with which to monitor the risks of e-cigarettes.

Table 6 Results for Precision (n = 6)

No.	Compound	0.1 mg/L		0.5 mg/L		2.0 mg/L	
		Retention Time RSD (%)	Peak Area RSD (%)	Retention Time RSD (%)	Peak Area RSD (%)	Retention Time RSD (%)	Peak Area RSD (%)
1	Formaldehyde	0.18	2.52	0.07	1.28	0.13	0.34
2	Acetaldehyde	0.19	3.36	0.09	0.86	0.08	0.27
3	Acetone	0.22	1.04	0.04	0.42	0.10	0.21
4	Acrolein	0.21	2.59	0.05	0.63	0.10	0.18
5	Propionaldehyde	0.21	4.29	0.05	1.31	0.09	0.19
6	Crotonaldehyde	0.10	0.48	0.04	0.35	0.05	0.10
7	2-butanone	0.10	4.10	0.03	0.53	0.04	0.51
8	Butyraldehyde	0.07	0.88	0.03	0.19	0.05	0.17

Table 7 Sample Analysis Results (mg/kg)

No.	Compound	Grapefruit, Mango, and Coconut Milk	Kiwi and Apple	Series B Strawberry Marshmallow	Mango	Icy Banana	Icy Lychee Berry	Limits
1	Formaldehyde	47.6	44.3	13.6	47.6	12.5	40.1	≤20
2	Acetaldehyde	44.6	83.7	22.2	6.0	31.7	33.2	≤180
3	Acetone	2.3	17.2	1.3	16.5	0.8	6.0	≤20
4	Acrolein	26.9	2.4	96.0	3.1	78.3	1.5	≤20
5	Propionaldehyde	N.D.	10.6	1.2	291.1	N.D.	N.D.	≤20
6	Crotonaldehyde	29.0	8.5	14.1	N.D.	5.5	6.6	≤20
7	2-butanone	N.D.	N.D.	N.D.	N.D.	0.9	1.5	≤20
8	Butyraldehyde	N.D.	7.5	N.D.	N.D.	N.D.	0.5	≤20

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