Don’t Let Formaldehyde “Hide” in Your Cosmetics! UHPLC-UV Detection Reveals the Truth About Formaldehyde Content

1. Introduction

Formaldehyde may be present in cosmetics, usually in trace amounts, primarily released from preservatives rather than added directly.According to regulatory requirements, the maximum permitted concentration of formaldehyde in cosmetics is 0.2%. While formaldehyde used within compliance limits poses a low carcinogenic risk, it can still cause allergic reactions in certain individuals.In addition, some toothpastes contain formaldehyde-releasing agents, which can slowly release formaldehyde under certain conditions. Although the amount is small, long-term use may still harm the body.Therefore, it is advisable to choose cosmetics and toothpastes with low or no formaldehyde content.

In this application, following the 2015 edition of the “Cosmetic Safety Technical Specification,” a certain brand of facial toner and toothpaste was analyzed using both pre-column derivatization and post-column derivatization methods, and the results of the two methods were compared.

2. Experimental Conditions

2.1 **Instruments and Equipment:** Leaps High-Performance Liquid Chromatograph

P60 Solvent Delivery Pump, A50CV Autosampler, C10V2 Column Oven, D10 UV Detector, R10 Post-Column Derivatization Device

2.2 Pre-Column Derivatization

2.3 **Preparation of Standard Solution:** Accurately measure an appropriate volume of formaldehyde standard solution into a 10 mL volumetric flask, dilute to volume with acetonitrile-water solution, and mix well to obtain a stock solution with a concentration of 1.04 mg/mL.

2.4 **Preparation of Standard Series Solutions:** Dilute the formaldehyde stock solution with acetonitrile-water to prepare standard series solutions of 1.3 μg/mL, 2.6 μg/mL, 5.2 μg/mL, 10.4 μg/mL, 52 μg/mL, 104 μg/mL, and 130 μg/mL. After preparation, take 1 mL of each formaldehyde standard solution, add 2 mL water, vortex for 2 minutes, centrifuge at 5000 r/min for 5 minutes, precisely pipette 1 mL of the supernatant into a 10 mL centrifuge tube, add 0.4 mL of 2,4-dinitrophenylhydrazine hydrochloride solution, vortex for 1 minute, let stand for 2 minutes, add 0.4 mL phosphate buffer solution, adjust to neutral pH with about 1.9 mL sodium hydroxide solution, vortex for 10 seconds, then add 4 mL chloroform, vortex for 3 minutes, centrifuge at 5000 r/min for 5 minutes, take 1 mL of the chloroform layer into a centrifuge tube, dry under nitrogen, dissolve

with 1 mL methanol, centrifuge at 5000 r/min for 5 minutes, and use the supernatant as the test solution.

2.5 **Sample Pretreatment:** Weigh 0.2 g (accurate to 0.0001 g) of toothpaste or cosmetic sample, dilute to 2 mL with acetonitrile–water solution, vortex for 2 minutes to mix well, centrifuge at 5000 r/min for 5 minutes, precisely pipette 1 mL of the supernatant into a 5 mL centrifuge tube, add 2 mL water, vortex for 30 seconds, centrifuge again if necessary (5000 r/min, 5 minutes), pipette 1 mL of the supernatant into a 10 mL centrifuge tube, add 0.4 mL 2,4–dinitrophenylhydrazine hydrochloride solution, vortex for 1 minute, let stand for 2 minutes, add 0.4 mL phosphate buffer, adjust to neutral pH with about 1.9 mL sodium hydroxide solution, vortex for 10 seconds, add 4 mL chloroform, vortex for 3 minutes, centrifuge at 5000 r/min for 5 minutes, take 1 mL of the chloroform layer into a centrifuge tube, dry under nitrogen, dissolve with 1 mL methanol, centrifuge at 5000 r/min for 5 minutes, and use the supernatant as the sample solution for testing.

3. Post-Column Derivatization

3. 1Preparation of Standard Solution: Accurately measure an appropriate volume of formaldehyde standard solution into a 10 mL volumetric flask, dilute to volume

with phosphoric acid solution, and mix well to obtain a stock solution with a concentration of 500 μg/mL.

3.2 **Preparation of Standard Series Solutions:** Prepare standard series solutions of formaldehyde at 1.0 μg/mL, 2.0 μg/mL, 5.0 μg/mL, 10 μg/mL, 20 μg/mL, and 50 μg/mL by diluting appropriate volumes of the stock solution with phosphoric acid solution.

3.3 **Sample Pretreatment:** Weigh 0.2 g (accurate to 0.0001 g) of toothpaste or cosmetic sample into a 10 mL stoppered colorimetric tube, dilute to the mark with phosphoric acid solution, vortex for 1 minute, centrifuge at 5000 r/min for 5 minutes, and filter the supernatant through a 0.45 μm aqueous filter membrane (if necessary, add 10 mL dichloromethane to the supernatant, shake, allow to separate, and then filter the aqueous layer through a 0.45 μm aqueous filter membrane).

4. Chromatographic Conditions

4.1 Pre-Column Derivatization

4.2 **Column**: Lotus C18 (4.6*250 mm, 5 μm)

Column Temperature: 30 ℃

Injection Volume: 10 μL

Wavelength: 355 nm

Mobile Phase A: Ultrapure water

Mobile Phase B: Methanol

4.3 Gradient Table for Pre-Column Derivatization:

时间 Time（min）	泵 Pump（mL/min）	A%	B%
0.0	1.0	90	10
12	1.0	10	90
15	1.0	90	10
20	1.0	90	10

4.4 Post-Column Derivatization;

Column：Lotus AQ C18（4.6*250 mm，5 μm）；

Column Temperature: 30 ℃;

Injection Volume: 10 μL;

Wavelength: 420 nm;

Mobile Phase: 0.2% phosphoric acid aqueous solution;

Flow Rate: 1.0 mL/min

5. Experimental Results

5.1 Chromatograms of Standard Solutions

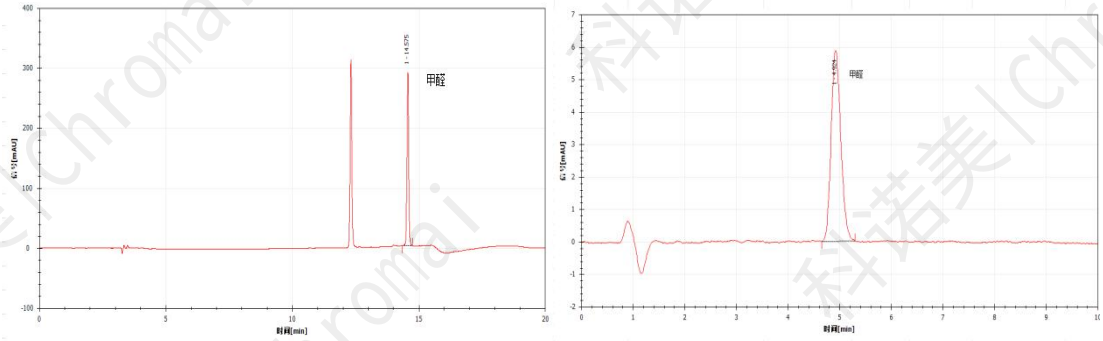

Figure 1 Chromatograms of standard solutions (Left: Pre-column derivatization; Right: Post-column derivatization)

5.2 Linearity

Standard solutions were injected in order from lowest to highest concentration. The concentration was used as the x-axis, and the peak area of the formaldehyde derivative at each concentration was used as the y-axis to plot a standard curve. Within the linear range, the correlation coefficient was greater than 0.9999.

Table 1 Results of Formaldehyde Standard Curves

Method	Compound	μg/mL (μg/mL)	Regression Equation	Correlation Coefficient
Pre-column derivatization	Formaldehyde	1.3~130.0	Y=9.7928X+2.6003	r=0.9999

Post-column derivatization	Formaldehyde	1.0~20.0	Y=54.753X-4.7544	r=0.9999

5.3 Repeatability

The same concentration of standard solution was injected six times. The RSD% for retention time was less than 0.07%, and the RSD% for peak area was less than 0.98%.

Table 2 Repeatability Results for Formaldehyde

Method	Compound	Retent. time RSD%	Peak Area RSD%
Pre-column derivatization	Formaldehyde	0.03	0.98
Post-column derivatization	Formaldehyde	0.07	0.80

5.4 Limit of Detection (LOD) and Recovery Rate

The LOD and recovery rate results for formaldehyde are shown in Table 3. The spiked recovery rate in toothpaste samples ranged from 100.56% to 103.00%.

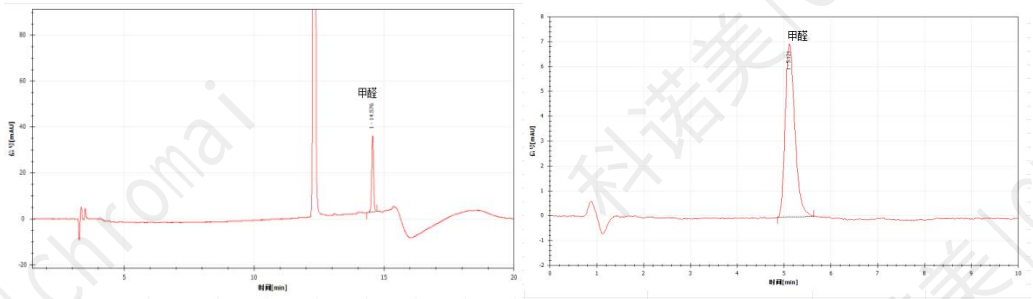

Figure 2 Chromatograms of Spiked Toothpaste Samples for Recovery Test (Left: Pre-Column Derivatization; Right: Post-Column Derivatization)

Method	Sample type	Test limit (mg/kg)	Recovery rate %
Pre-column derivatization	Toothpaste	10	100.56
	Cosmetic water	10	101.79
Post-column derivatization	Toothpaste	30	103.00
	Cosmetic water	30	101.20

6. Conclusion

In this application, the Konomei Leaps UHPLC－UV detector was used to determine formaldehyde content via pre-column and post-column derivatization methods. Both methods showed good linearity with correlation coefficients greater than 0.9999.For real samples, the recovery rates for toothpaste using

both derivatization methods ranged from 100.56% to 103.00%, while the recovery rates for cosmetic water ranged from 101.20% to 101.79%.The LODs for pre-column and post-column derivatization were 10 mg/kg and 30 mg/kg, respectively, both meeting the requirements of the "Cosmetic Safety Technical Specification."The pre-column derivatization method had a lower LOD due to better derivatization conditions and reagent selection, but required manual derivatization, making it more prone to human error.The post-column derivatization method was more automated and provided more stable detection results.