Simultaneous Determination of α-Arbutin, β-Arbutin, Deoxyarbutin, Hydroquinone, and Phenol in Cosmetics by Liquid Chromatography-Fluorescence Detector

1. Foreword

Arbutin appears as white needle - like crystals or powder. It can inhibit the activity of tyrosinase in the body, prevent the formation of melanin, and has a good whitening effect. Therefore, it is often used in whitening cosmetics. α - Arbutin and β - arbutin are isomers with different whitening effects, and both can be converted into hydroquinone under certain conditions. Deoxyarbutin has a strong antioxidant effect and can also effectively inhibit the activity of tyrosinase. It can be converted into hydroquinone under room temperature, weak acid conditions, or when exposed to light in an aqueous solution. The European Commission's Regulation (EU) 2021/1099 prohibits the use of deoxyarbutin in cosmetics. Hydroquinone and phenol also have good whitening effects, but due to their high toxicity and strong irritation, long - term exposure may pose a carcinogenic risk. In China's "Technical Specifications for Cosmetic Safety" (2015 Edition), both are listed as prohibited raw materials for cosmetics. Therefore, it is necessary to establish a method for the simultaneous determination of these five compounds.

Currently, the detection methods for these compounds mainly include micellar electrokinetic capillary chromatography, high - performance liquid chromatography - diode array detection, gas chromatography, gas chromatography - tandem mass spectrometry, etc. Usually, only 1-3 of these substances can be detected, and deoxyarbutin has not been detected. This application simultaneously analyzes these five compounds by high -

performance liquid chromatography - fluorescence detector.

2. Experimental Conditions

2.1 Instruments：UHPLC（P40 - Quaternary gradient pump、A10 - Autosampler、C10 - Column Oven、D50 - Flourescence detector）

3. Chromatographic Conditions

3.1 **Chromatographic column:** Lotus C18，5μm，4.6*250 mm

3.2 **Column temperature:** 25 ℃

3.3 **Injection volumen:** 10 μL

3.4 **Detection wavelength:**

Compound	Excitation wavelength	Emission wavelength
α-Arbutin	292	337
β-Arbutin	292	337
Deoxyarbutin	293	322
Hydroquinone	298	345
Pheno	280	318

3.4.1 Mobile phase A：Methanol; Mobile phase B：Water;

3.4.2 Gradient program：

Time（min）	Flow rate（mL/min）	A%	B%
0.0	0.5	10	90
11.0	0.5	10	90

11.1	1.0	15	85
30.0	1.0	80	20
30.1	1.0	10	90
40.0	1.0	10	90

4. Experimental results

4.1 System compliance

As can be seen from Figure 1 (the chromatogram of the standard solution), the five compounds are well - separated. The resolution between α - arbutin and β - arbutin reaches 1.775, achieving baseline separation.

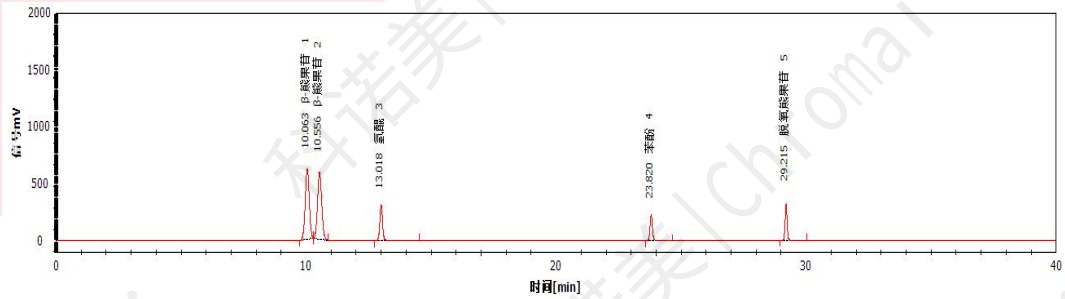

Figure 1 Chromatogram of the standard solution

(α - Arbutin and β - arbutin 1.0 μg/mL, deoxyarbutin, hydroquinone, and phenol 0.2 μg/mL)

Table 1 System Suitability Results Table

Compund	Concentration（μg/mL）	Retention time	Tailing factor	Column efficiency	Resolution
α - Arbutin	1.0	10.267	1.030	20849.043	-
β - Arbutin	1.0	10.784	1.013	20959.403	1.775
Deoxyarbutin	0.2	13.224	1.042	72694.007	9.871

Hydroquinone	0.2	23.946	1.054	307496.985	58.11
Phenol	0.2	29.293	1.054	632357.618	33.396

4.2 Linearity and sensitivity

As shown in Table 2, α - arbutin and β - arbutin have a good linear relationship in the concentration range of 0.5 - 50 μg/mL, and deoxyarbutin, hydroquinone, and phenol have a good linear relationship in the concentration range of 0.1 - 10 μg/mL.

Table 2 Linear and Detection Limit, Quantitation Limit Results of Five Compounds

Compund	Concentration（μg/mL）	Regression equation	Correlation coefficient	LOD（ng/mL）	LOQ (ng/mL)
α - Arbutin	0.5~50	Y=138.36X+10.349	r=1.0000	3.08	10.25
β - Arbutin	0.5~50	Y=142.91X+12.460	r=1.0000	3.28	10.95
Deoxyarbutin	0.1~10	Y=185.31X-8.1111	r=0.9999	1.54	5.13
Hydroquinone	0.1~10	Y=214.01X-12.108	r=0.9999	1.84	6.14
Phenol	0.1~10	Y=134.76X+1.2816	r=1.0000	1.43	4.76

4.3 Repeatability

Three standard solutions with different concentrations were injected 6 times respectively, and the RSD% of the peak area and retention time of each compound was calculated. The results are shown in Table 3. At the three concentration levels, the RSD% of the retention time of each compound is less than 0.2%, and the RSD% of the peak area is less than 0.4%.

Table 3 Repeatability Results of Five Compounds

Concentration level	Retention time RSD%		Peak area RSD%	
	α - Arbutin	β - Arbutin	α - Arbutin	β - Arbutin
0.5 μg/mL	0.15%	0.10%	0.24%	0.21%

10 μg/mL	0.12%		0.09%	0.21%		0.16%
50 μg/mL	0.06%		0.07%	0.07%		0.09%
Concentration level	Retention time RSD%			Peak area RSD%		
	Deoxyarbutin	Hydroquinone	Phenol	Deoxyarbutin	Hydroquinone	Phenol
0.1 μg/mL	0.02%	0.08%	0.03%	0.35%	0.39%	0.36%
2 μg/mL	0.05%	0.05%	0.05%	0.29%	0.26%	0.22%
10 μg/mL	0.01%	0.02%	0.02%	0.31%	0.14%	0.31%

4.4 Spiked recovery

α - Arbutin and β - arbutin were added to the facial cream sample at a concentration of 20 μg/kg, and deoxyarbutin, hydroquinone, and phenol were added at a concentration of 4 μg/kg. The spiked recovery results were 95.7%, 96.0%, 90.1%, 98.1%, and 93.9% respectively.

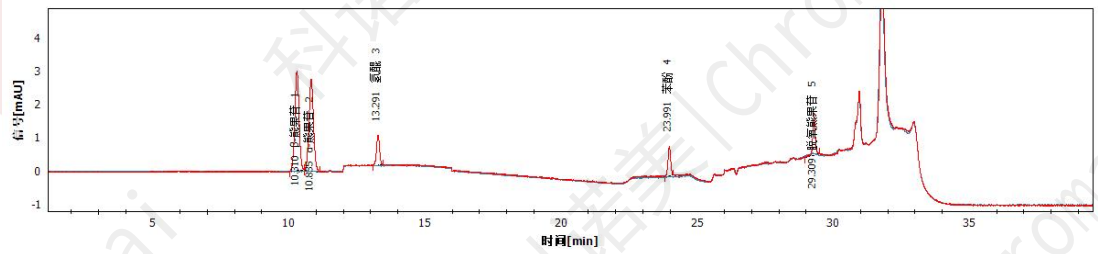

Figure 2 Chromatograms of the blank sample (blue) and the spiked sample (red)

5. Conclusion

This method uses Chromai's UHPLC - fluorescence detector to simultaneously determine the contents of α - arbutin, β - arbutin, deoxyarbutin, hydroquinone, and phenol in cosmetics. The method has a good linear relationship. The RSD% of the retention time of each compound is less than 0.2%, and the RSD% of the peak area is less than 0.4%. There is no interference at the target peak of the actual sample (facial cream). The spiked

recovery of the actual sample is 93.9% - 98.1%. The detection limit and quantitation limit meet the detection requirements.