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"An efficient analytical method for evaluating the high quality of Bakuchiol in cosmetics industry: HPLC combined with normalization method"

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1. Introduction

Bakuchiol is a natural ingredient isolated from the seeds of *Psoralea corylifolia*. It is an emerging ingredient with promising retinoid-like anti-aging properties, along with anti-microbial, hyperpigmentation, and anti-cancer activities [1][2][3]. With the standardization of China's cosmetics regulations and the implementation of new raw material filing regulations, *bakuchiol* has emerged as a new introduced ingredient and a promising candidate for inclusion in Catalog of Used Cosmetic Raw Materials. This trend catalyzed growing industry interest, with an increasing number of cosmetic brands and manufacturers actively exploring bakuchiol's commercial potential as a novel cosmetic component [4]. The large-scale acquisition of bakuchiol component is usually achieved through natural plant extraction from the seeds of *Psoralea corylifolia* L. [5], which inevitably leads to the coexistence of many impurities. In addition, its use is still controversial due to the presence of these phototoxic agents (psoralen, isopsoralen and coumarin derivatives) [1][6]. Nowadays, there are still gaps and deficiencies in the quality standards of bakuchiol in the market [4]. At present, official regulation and standards are lack of comprehensive quality assessment system to bakuchiol and detailed restriction to the kind of impurities and limitations have not been declared yet [10]. Notably, the current quality evaluation methods for bakuchiol raw material are often one-sided, evaluating the impurity content or the content of bakuchiol compound unilaterally, lacking a comprehensive evaluation method [7][8][10]. Therefore, we obtained a composite quality score through the normalization of the data, and directly compared the composite quality score to make an intuitive comparison of raw materials which can eveluate the quality of backuchiol.

In our previous study, we optimized the test concentrations suitable for bakuchiol and impurity analysis separately as we noticed the trace nature of the impurities. In this work, the contents of bakuchiol, psoralen, isopsoralen, neobavaisoflavone and bavachin were determined by HPLC external standard method. The purity data was normalized so that the data is distributed between 0-1. Then the composite quality score was obtained through data processing, and the comprehensive quality of raw materials was evaluated through the score ranking.

2. Materials and Methods

2.1. Chemicals and materials

High-performance liquid chromatography (HPLC)-grade acetonitrile and methanol were purchased from Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). High purity deionized water (18.2 MΩ·cm) was generated using an Arium® Comfort I water purification system (Sartorius AG, Göttingen, Germany). Bakuchiol samples were collected from seven suppliers in China (Table 1).

Table 1. Details of the bakuchiol materials collected.

Sample	Lot	Area
A1	20231214	China
A2	B*240201	China
A3	W*240401	China
A4	20241016	China
B1	H*0419	China
B2	24062501	China
B3	20240607	China

Psoralen (purity ≥ 99%), isopsoralen (purity ≥ 99%), neobavaisoflavone (purity ≥ 99%), and bavachin (purity ≥ 98%) were obtained from Chengdu Gelipu Biotechnology Co., Ltd. (Sichuan, China). Bakuchiol reference standard (purity ≥ 98%) was acquired from Adamas-beta Co., Ltd. (Shanghai, China).

2.2. Sample preparation

Seven samples were prepared in methanol at two concentration levels: 0.5 mg/mL for purity assessment and 250 mg/mL for impurity analysis, respectively. The calibration solutions were systematically generated using methanol serial dilution. Working standards for quantitative analysis of bakuchiol were established across a linear range of 0.2-0.8 mg/mL. Four characteristic impurities (psoralen, isopsoralen, neobavaisoflavone, and bavachin) were measured simultaneously using a mixed calibration curve in the 0.25-5 ppm range. All solutions were filtered through a 0.45 μm membrane before injection.

2.3. HPLC analysis for impurity in bakuchiol

The analysis method was slightly modified according to Chen et. al [11].The chromatography analysis was performed using an Agilent 1260 Infinity Ⅱ HPLC system (Agilent Technologies, CA, USA) equipped with a diode array detector (DAD) and a reversed-phase C18 column (150 × 4.60 mm, 5 μm) (Agilent Technologies, CA, USA). The gradient elution program employed mobile phase A (pure water) and B (acetonitrile) under the following conditions: 0–15 min, 35% B; 15–15.1 min, 35%→45% B; 15.1–32 min, 45% B; 32–32.1 min, 45→95% B; 32.1–37 min, 95% B; 37–37.1, 95→35% B; 37.1–42，35% B, with a constant flow rate of 1.0 mL/min. The column temperature was maintained at 30°C with a 10-μL injection volume. The DAD detection spanned 200 to 600 nm, with quantitative integration performed at 246 nm. Chromatograms as well as absorption spectra in the UV were obtained for each component using HPLC.

2.4 HPLC analysis for purity of bakuchiol

Purity analysis was conducted on the same HPLC system as before, with isocratic elution (acetonitrile / water, 80:20, V/V) for 12 minutes. Chromatographic separation was obtained at 30°C column temperature with a mobile phase flow rate of 1.0 mL/min and injection volume of 10 μL . The DAD was set to monitor at 262 nm.

2.5 Data processing

1) Calculation of bakuchiol purity in the sample

The bakuchiol content of the sample to be tested was calculated based on the standard curve. The purity (%) was calculated using the following equation:

$x = (C / 0.5) \times 100\%,$

where C represents the experimentally determined bakuchiol concentration (expressed in mg/mL) from the calibration curve, and 0.5 denotes the theoretical concentration (0.5 mg/mL) of the test solution.

2) Normalization of purity values

The measured purity values (x) were scaled to a 0-1 normalized range (x‘) using min-max normalization:

$x' = [x - x_{min}] / [x_{max} - x_{min}],$

where x_{min} and x_{max} represent the minimum and maximum purity values observed across the seven tested samples, respectively.

3) Composite quality score calculation

A quality assessment metric (*y'*) was calculated through:

$y' = (x' / y) \times 100\%,$

where *x'* denotes the normalization value of sample, *y* denotes the total impurity content (expressed in ppm). Higher *y'* values reflecting superior sample quality through synergistic evaluation of both purity and impurity content.

2.6 Statistical analysis

All experiments were run in triplicate. The data were presented as the mean ± standard. Graphical representations were generated using Graph Pad Prism 9 (v.9.0.0; GraphPad Software, San Diego, CA) for data visualization and statistical analysis.

3. Results

3.1 Identification of bakuchiol, psoralen, isopsoralen, neobavaisoflavone, and bavachin

Qualitative analysis of bakuchiol and four impurities in the sample was carried out using HPLC based on the spectrum and retention time. The chromatograms and UV absorption spectra of the standard and sample were shown in Figure 1.

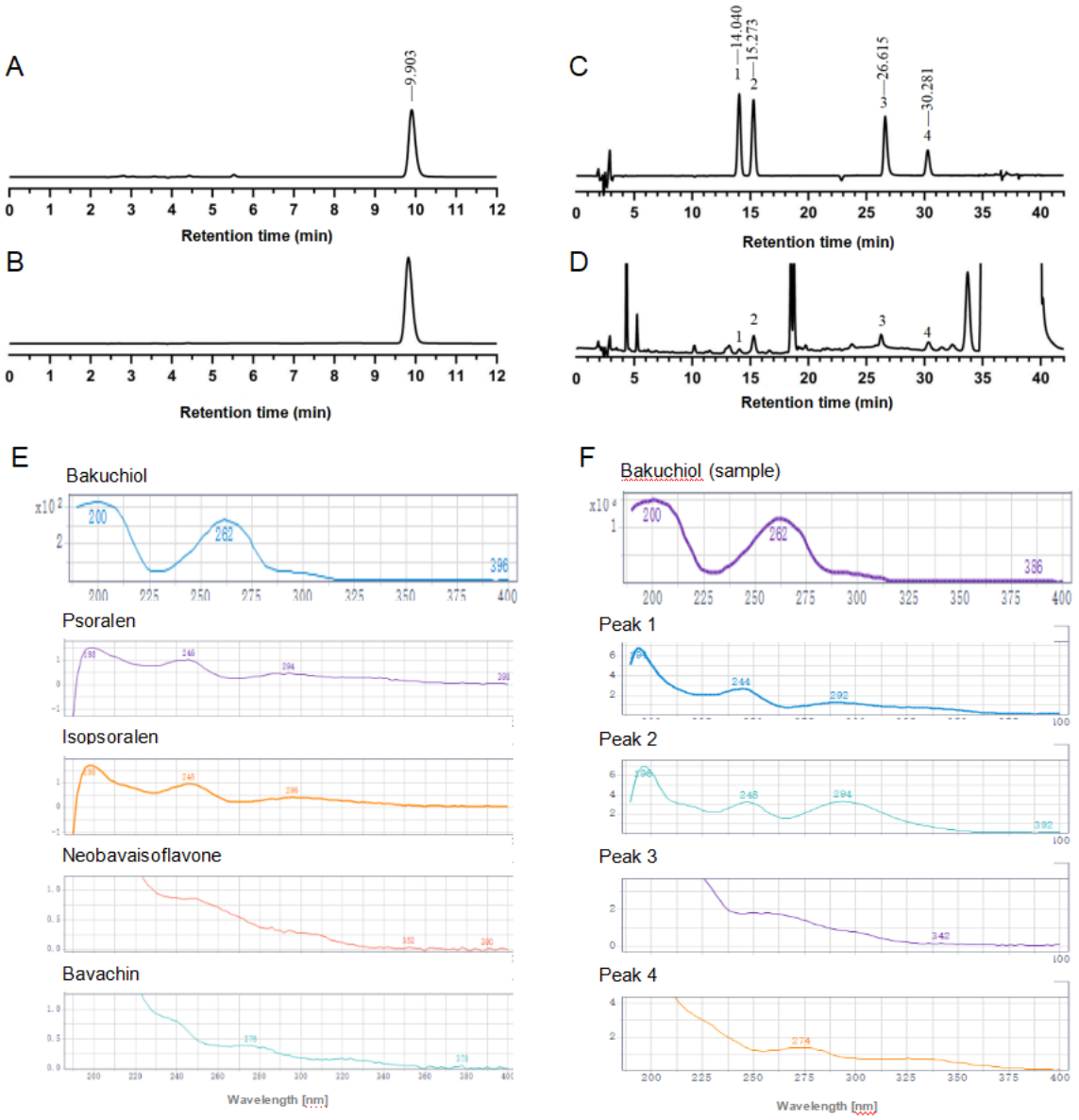

Figure 1. Chromatograms of standard bakuchiol (A) and sample (B). Typical chromatograms of 4 standard analytes (C) and sample (D) by HPLC-UV. Peak identification: 1 psoralen, 2 isopsoralen, 3 neobavaisoflavone, 4 bavachin. The UV absorption spectra of the identified peaks of standards (E) and sample (F).

3.2 The purity and impurity content of bakuchiol samples

The HPLC-UV analysis revealed significant variations in bakuchiol purity across the seven tested samples (Table 2). The quantified purity levels spanned from 82.95% (Sample B2) to 114.83% (Sample A2), with three samples (A2, A3, A4) exceeding 110% purity.

Table 2. The contents of psoralen, isopsoralen, neobavaisoflavone, bavachin (ppm) and bakuchiol purity (%) of 7 bakuchiol samples by HPLC-UV.

Sample	Content (ppm)					Purity（%）
	psoralen	isopsoralen	neobavaisoflavone	bavachin	Total impurities	

A1	3.62±0.05	1.93±0.00	N.D.	4.17±0.06	9.72	104.98±0.72
A2	2.72±0.05	5.02±0.07	N.D.	0.19±0.01	7.94	114.83±2.62
A3	3.39±0.01	15.56±0.02	N.D.	13.08±0.40	32.03	113.86±6.44
A4	1.92±0.03	4.25±0.16	2.32±0.42	1.82±0.78	10.31	112.33±7.10
B1	4.72±0.10	4.20±0.11	N.D.	3.09±0.14	12.01	99.19±21.21
B2	4.81±0.04	32.64±0.12	N.D.	0.36±0.62	37.81	82.95±5.29
B3	3.96±0.11	6.57±0.21	N.D.	N.D.	10.52	111.24±4.53

Note: N.D. = Not detected.

Total impurity content exhibited pronounced differences, ranging from 7.94 ppm (Sample A2) to 37.81 ppm (Sample B2). This difference in total impurities primarily stemmed from variable concentrations of isopsoralen (1.93-32.64 ppm) and bavachin (0.19-13.08 ppm), with psoralen content remaining relatively stable (1.92-4.81 ppm). Particularly noteworthy was Sample B2, which demonstrated both the highest isopsoralen concentration (32.64 ± 0.12 ppm) and the lowest purity value (82.95 ± 5.29%).

3.3 Composite quality score

Normalized purity and comprehensive score are summarized in Table 3.

Table 3. Comprehensive evaluation results of 7 bakuchiol samples

Sample	Normalization of purity data	Total impurity content	Composite quality score/%
A1	0.69	9.72	7.13%
A2	1.00	7.94	12.60%
A3	0.97	32.03	3.03%
A4	0.92	10.31	8.94%
B1	0.51	12.01	4.25%
B2	0.00	37.81	0.00%
B3	0.89	10.52	8.43%

Through data processing, the comprehensive score is distributed in 0-12.60%, and the raw materials of 7 suppliers are ranked: A2 > A4 > B3 > A1 > B1 > A3 > B2 (Table 3). Supplier A2 achieved the highest score (12.60%) while B2 scored lowest (0.00%).

Notably, the top-ranked Sample A2 exhibited exceptional quality parameters with 1.15-fold purity than the control (114.83% vs nominal 100%) coupled with minimal impurity burden (7.94 ppm total). Conversely, the lowest-performing Sample B2 showed critical quality deficiencies characterized by 17% purity lower than the control (82.95%) and the highest impurities (37.81 ppm). The inverse correlation between total impurities and comprehensive scores confirmed the robustness of this evaluation metric.

4. Discussion

This study successfully developed a systematic method coupled with HPLC purity analysis, impurity quantification, data normalization, and a composite scoring algorithm, providing a novel tool for evaluating the quality of bakuchiol raw materials in the cosmetics industry. In our work, we conducted quantitative analysis on four impurities and bakuchiol of seven bakuchiol samples in the market. Analysis of samples from seven suppliers revealed significant variations in both purity (82.95–114.83%) and total impurity content (7.94–37.81 ppm). On the one

side, we could obtain a roughly rank according to purity value as follows: A2 > A3 > A4 > B3 > A1 > B1 > B2. On the other side, we could get another ranking according to the total impurity content as follows: A2 > A1 > A4 > B3 > B1 > A3 > B2. It is obviously that Sample A2 could be identified as the highest quality sample due to its low phototoxic impurity levels and high purity. Similiarly, Sample B2, identified as the lowest quality due to its low purity (82.95%) and high phototoxic impurity levels (e.g., 32.64 ppm isopsoralen) as well. However, there is no consistent result in purity and the phototoxic impurity ranking among the other 5 samples, which underscores the limitations of relying solely on purity or impurity quantitative assessment. We designed a comprehensive evaluation system that integrates the two indicators to comprehensively evaluate the overall quality of raw materials in a more intuitive way. The composite quality of the samples was expressed in the way of comprehensive scores percent in 0-12%, and based on the score, the ranking of the samples was as follows: A2 > A4 > B3 > A1 > B1 > A3 > B2 (Table 3). Therefore, the composite scoring system effectively integrates both purity and impurity metrics, offering a more holistic evaluation of raw material safety.

The proposed method holds significant practical value for the cosmetics industry. By normalizing data and calculating composite scores, companies can objectively compare raw materials from different suppliers, prioritizing high-purity, low-impurity options (e.g., A2) to mitigate phototoxic risks. This aligns with China's updated cosmetics regulations, which emphasize ingredient safety. Furthermore, the framework serves as a reference for quality evaluation of other natural ingredients, particularly those requiring multi-impurity analysis.

However, this study has limitations. The small sample size (seven suppliers) may not fully represent market diversity. Additionally, the analysis focused on four known phototoxic impurities, omitting other potential associated impurities, as these samples were obtained from complex natural plant extracts [9]. Future research should expand the sample scope to include more suppliers and geographical sources, broaden impurity detection categories, and validate the method's applicability across diverse chromatographic conditions or instruments to enhance its universality.

5. Conclusion

Bakuchiol is a new ingredient in the dermo-cosmetic market. Numerous studies have shown that bakuchiol has an anti-aging effect. However, with a large amount of bakuchiol raw materials entering the market, it is necessary to evaluate and regulate their overall quality. This study established an efficient and systematic quality evaluation method for bakuchiol raw materials using HPLC combined with data normalization and a composite scoring algorithm. The results demonstrated marked variability in purity and impurity levels among suppliers, with the scoring system effectively distinguishing high-quality materials (e.g., Sample A2) from high-risk ones (e.g., Sample B2). This approach not only provides cosmetics manufacturers with a scientific tool for raw material screening but also supports compliance with China's evolving regulatory standards for ingredient safety. Future efforts should focus on expanding detection parameters, increasing sample diversity, and adapting this methodology to other natural ingredients to advance the cosmetics industry toward safer and more standardized practices.

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Conflict of Interest Statement

There are no conflicts to declare.

Reference

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