Research on Quality Standards for Pomelo (*Citrus maxima*) Peel

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Abstract [**Objectives**] To establish a quality standard for pomelo (*Citrus maxima*) peel. [**Methods**] According to the relevant methods specified in the General Chapters of the *Chinese Pharmacopoeia* (2020 Edition, Volume IV), ten batches of pomelo peel from different producing areas were examined for moisture, total ash, acid-insoluble ash, and extractives. A thin-layer chromatography (TLC) identification method was established using naringin as the marker, and high-performance liquid chromatography (HPLC) was employed for the content determination of naringin. [**Results**] Microscopic examination revealed characteristic structures of the *Citrus* genus, such as calcium oxalate prism crystals, vessels, and oil cells. The TLC spots were clear and well separated without interference from the negative control. For the ten batches of samples, the moisture content ranged from 9.8% to 12.7%, total ash from 3.1% to 5.1%, acid-insoluble ash from 0.04% to 0.2%, water-soluble extractives from 44.1% to 57.2%, ethanol-soluble extractives from 31.4% to 44.6%, and naringin content from 1.65% to 7.63%. [**Conclusions**] The established quality standard method is accurate, reliable, and reproducible, and can be applied to the quality control of pomelo peel.

Key words Pomelo (*Citrus maxima*) peel, Naringin, Thin-layer chromatography (TLC) identification, Quality standard, High-performance liquid chromatography (HPLC)

1 Introduction

Pomelo peel, the fruit peel of *Citrus maxima* (Burm.) Merr. (Rutaceae, *Citrus*), constitutes about 30% of the total fruit weight[1]. Modern pharmacological research has revealed that it contains flavonoids (*e. g.*, naringin, naringenin, tangeretin, and quercetin) and coumarin derivatives. Naringin, the predominant constituent[1], possesses multiple bioactivities, including antioxidant, anti-inflammatory, hypolipidemic, and immunomodulatory effects[2-3]. Value-added processing of pomelo peel can produce a range of high-value products and ingredients for the food, cosmetic, and pharmaceutical sectors. However, current exploitation has mainly focused on food processing and its applications[4]; systematic quality assessment of pomelo peel as a medicine-food homologous material remains insufficient. In particular, well-established quality standards covering standardized testing parameters and marker-based assay methods are still lacking. Therefore, systematic pharmacognostic and quality evaluation studies are urgently needed to facilitate the rational utilization of this resource. The present study, carried out in accordance with the General Chapters of the *Chinese Pharmacopoeia* (2020 Edition, Volume IV)[5], evaluated the macroscopic and microscopic features, TLC profiles, moisture, total ash, and extractives of pomelo peel. In addition, a quantitative assay using naringin as the representative bioactive marker was developed. This work aims to provide scientific evidence for the authentication, quality standardization, and comprehensive exploitation of pomelo peel.

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2 Materials and methods

2.1 Materials and Reagents The pomelo peel samples were collected from different regions of China (Table 1). The reference crude drug (YZP-11) was authenticated as the fruit peel of *Citrus maxima* (Burm.) Merr. by Professor Zhou Xiaolei from Guangxi Botanical Garden of Medicinal Plants. Naringin (Lot. MUST-25051902) was purchased from Chengdu MUST Biotechnology Co., Ltd. Methanol (HPLC grade, Lot. F24O5K23) was obtained from Fisher Chemical. Wahaha purified water was used. Silica gel G TLC plates (20 cm × 10 cm) were purchased from Shandong Yantai Xinnuo Company.

Table 1 Sample collection information of pomelo peel

Batch No.	Production area	Collection time
YZP-1	Rongxi Town, Rongxian County, Yulin City, Guangxi	2023 - 11 - 23
YZP-2	Ziliang Town, Rongxian County, Yulin City, Guangxi	2023 - 11 - 22
YZP-3	Luojiang Town, Rongxian County, Yulin City, Guangxi	2023 - 11 - 22
YZP-4	Luojiang Town, Rongxian County, Yulin City, Guangxi	2023 - 11 - 22
YZP-5	Xiandi Town, Rongxian County, Yulin City, Guangxi	2023 - 11 - 24
YZP-6	Lingjing Town, Tengxian County, Wuzhou City, Guangxi	2023 - 11 - 22
YZP-7	Lingao County, Hainan Province	2023 - 10 - 08
YZP-8	Pinghe County, Zhangzhou City, Fujian Province	2023 - 10 - 08
YZP-9	Meizhou City, Guangdong Province	2023 - 10 - 10
YZP-10	Luzhou City, Sichuan Province	2023 - 10 - 12

2.2 Identification

2.2.1 Macroscopic and microscopic identification. After drying the freshly harvested pomelo peel, intact samples free of insect holes were selected and their macroscopic characteristics were

recorded. Subsequently, the pomelo peel was pulverized, and the powder was passed through a No. 6 sieve. A small amount of the powder was mounted with dilute glycerol, and a separate preparation was made using chloral hydrate for clearing. Observations were performed under an inverted microscope (IX73, OLYMPUS).

2.2.2 TLC Identification. Preparation of the test solution: A 1.0 g portion of pomelo peel powder was mixed with 20 mL of ethanol, sonicated for 30 min, and filtered through a 0.45 μm membrane filter to yield the test solution. Preparation of the reference solution: Naringin reference standard was accurately weighed and dissolved in methanol to prepare a solution with a concentration of 1.0 mg/mL. TLC identification was performed using a UV analyzer (Model ZF-8). The test solution (2 μL) and the reference solution (2 μL) were spotted onto the same TLC plate. A mixture of ethyl acetate-methanol-water (8 ∶ 2 ∶ 2, *v/v/v*) was used as the developing solvent. Development was carried out at 25 ℃ with a relative humidity of approximately 32%, over a distance of about 8 cm. After removal, the plate was dried in air and examined under 365 nm UV light for fluorescent spots. The plate was then sprayed with 10% sulfuric acid solution, heated at 100 ℃ for 3 min until the spots were clearly colored, and examined under visible light.

2.3 Inspection

2.3.1 Moisture. The moisture content of ten pomelo peel samples was determined in triplicate by the oven-drying method (Method 2) under Item 0832 of the General Chapters of the *Chinese Pharmacopoeia* (2020 Edition, Volume IV). The data were calculated according to Equation (1):

$$\mu = \overline{X} + \frac{ts}{\sqrt{n}} + MU \quad (1)$$

where μ is the limit value; $\overline{X}$ is the mean value; t is the critical value from the t-distribution table, 2.821; s is the standard deviation; n = 10; the expanded uncertainty of moisture, U is 0.127 0; and MU is the product of the mean value and the expanded uncertainty.

2.3.2 Total ash and acid-insoluble ash. The total ash and acid-insoluble ash of the ten samples were determined according to the method described under Item 2302 of the General Chapters of the *Chinese Pharmacopoeia* (2020 Edition, Volume IV). The expanded uncertainty (U) for total ash was 0.052 8, calculated using Formula (1) in Section **2.3.1**.

2.3.3 Extractives. The extractives of the ten samples were determined using water and 70% ethanol as solvents, following the hot extraction method described under Item 2201 of the General Chapters of the *Chinese Pharmacopoeia* (2020 Edition, Volume IV). The results were calculated according to Equation (2):

$$\mu = \overline{X} + \frac{ts}{\sqrt{n}} - MU \quad (2)$$

The expanded uncertainty (U) of extractives was 0.128 8, and the explanations of other parameters were the same as that in Section **2.3.1**.

2.4 Content determination

2.4.1 Preparation of the test solution. Approximately 0.2 g of pomelo peel powder was accurately weighed and placed in a stoppered conical flask. Then, 50 mL of 70% methanol was accurately added. The flask was tightly stoppered and weighed. After sonication (power 500 W, frequency 40 kHz) for 30 min, the flask was weighed again. The weight loss was replenished with 70% methanol, and the mixture was shaken well and filtered through a 0.45 μm microporous membrane to obtain the test solution.

2.4.2 Preparation of the reference solution. An accurately weighed 5 mg portion of naringin reference standard was dissolved in 70% methanol and diluted to 10 mL to obtain a solution with a concentration of 0.5 mg/mL. Aliquots of 0.050, 0.100, 0.250, 0.500, 1.000, and 5.000 mL of this solution were accurately transferred to separate 10 mL volumetric flasks, diluted to volume with 70% methanol, and mixed well. The peak areas were measured under the chromatographic conditions, and a calibration curve was constructed by plotting the peak areas against the corresponding concentrations.

2.4.3 HPLC conditions. Chromatographic separation was carried out on a ShimNex CS C_{18} column (4.6 mm × 250 mm, 5 μm) with a mobile phase consisting of methanol (A) and 0.1% phosphoric acid in water (B). Gradient elution was programmed as follows: 0 – 10 min, 40% →25% A; 10 – 15 min, 25% →45% A; 15 – 25 min, 45% →55% A; 25 – 35 min, 55% →50% A. The detection wavelength was set at 283 nm, the column temperature was maintained at 30 ℃, the injection volume was 10 μL, and the flow rate was 1.0 mL/min.

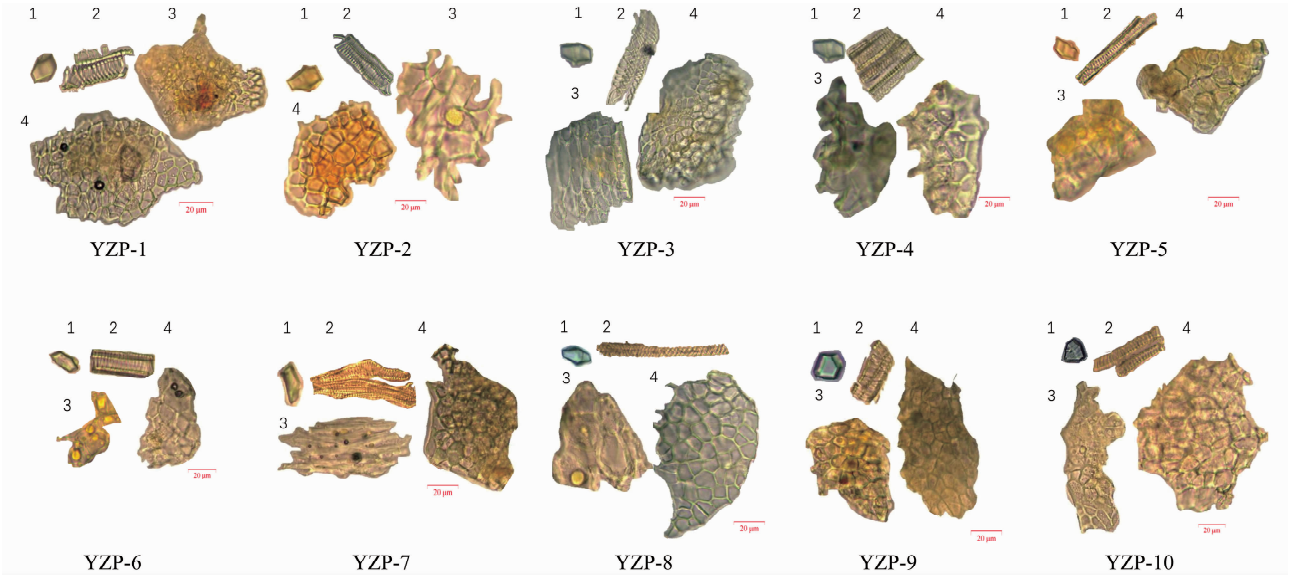
3 Results and analysis

3.1 Identification

3.1.1 Macroscopic identification. After drying, the margins of the pomelo peel pieces curled slightly inward. The outer surface was yellowish-green to golden yellow, occasionally yellowish-brown, and exhibited numerous sunken dots and raised oil points. The inner surface was white, somewhat soft and elastic, and cotton-like in texture. The peel was 0.5 – 2.5 cm thick, pliable, and possessed a strong pomelo fragrance.

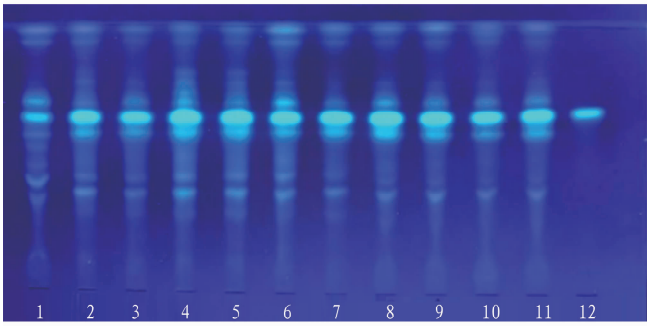
3.1.2 Microscopic identification. The powder appeared pale yellow or yellowish-brown. Oil cavities (secretory cavities), vessels, parenchyma cells, and calcium oxalate crystals were detected in all samples. Calcium oxalate prisms, characteristic crystals of the Citrus genus in the Rutaceae family, bordered-pitted vessels as typical structures of vascular tissue, and oil cavity cells as storage structures for volatile oils together constituted the microscopic identification system for pomelo peel (Fig. 1).

3.1.3 TLC identification. TLC examination demonstrated that all ten batches of the crude drug produced fluorescent spots of the same color at the positions corresponding to the reference substance (Figs. 2 and 3), with good resolution, appropriate *Rf* values, and satisfactory reproducibility.



NOTE 1. Calcium oxalate prism crystals; 2. Vessels; 3. Oil cavity cells; 4. Mesocarp parenchyma cells.

Fig. 1 Powder micrographs of 10 batches of pomelo peel



NOTE 1 – 10: Ten batches of pomelo peel samples; 11: Reference crude drug; 12: Naringin reference standard.

Fig. 2 TLC fluorescence chromatograms of 10 batches of pomelo peel samples (365 nm)

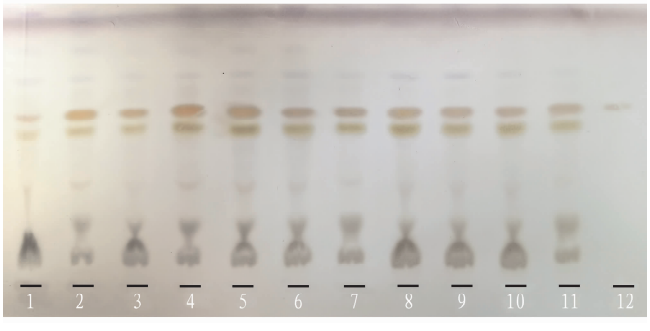


Fig. 3 TLC chromatograms under visible light of ten batches of pomelo peel samples (visualized with 10% sulfuric acid followed by heating)

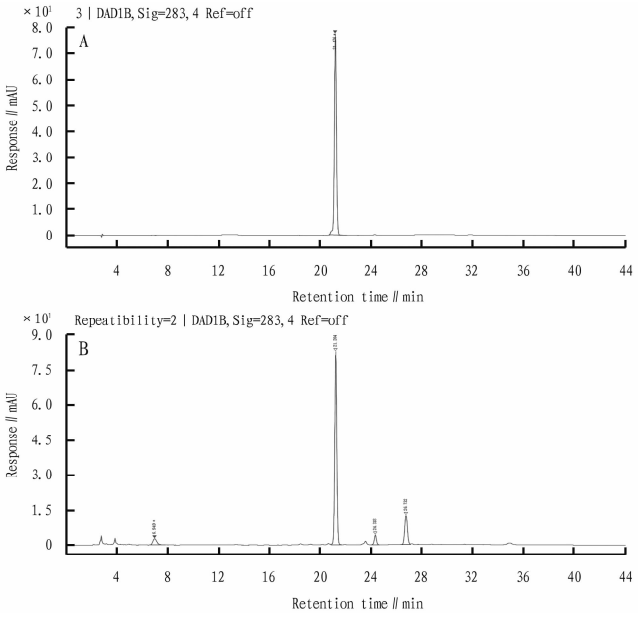
3.2 Inspection Results (Table 2) showed that the moisture content of ten batches of pomelo peel from different producing areas ranged from 9.8% to 12.7%, with an overall mean of 10.98%. The total ash content ranged from 3.1% to 5.1%, with an overall mean of 3.85%; the acid-insoluble ash content ranged from 0.04% to 0.2%, with an overall mean of 0.09%. The water-soluble extractives content ranged from 44.1% to 57.2%, with an average of 50.41%, and the ethanol-soluble extractives content ranged from 31.4% to 44.6%, with an average of 38.14%. Based on the calculations using formulas (1) and (2), the proposed quality limits for pomelo peel were as follows: moisture ≤14.0% (μ = 13.26%), total ash ≤5% (μ = 4.60%), acid-insoluble ash ≤0.2% (μ = 0.15%), water-soluble extractives ≥45% (μ = 48.46%), and ethanol-soluble extractives ≥ 35% (μ = 37.35%).

Table 2 Results of moisture, total ash, acid-insoluble ash, and extractives of pomelo peel (%, n = 3)

Batch No.	Moisture	Total ash	Acid-insoluble ash	Water-soluble extractives	Ethanol-soluble extractives
YZP-1	10.10	3.70	0.06	53.40	42.10
YZP-2	10.30	3.70	0.00	45.00	36.00
YZP-3	11.60	3.50	0.04	50.60	40.60
YZP-4	10.90	4.30	0.10	44.10	33.10
YZP-5	10.50	3.10	0.20	54.40	44.00
YZP-6	12.70	3.30	0.04	56.20	39.30
YZP-7	9.80	3.50	0.08	57.20	44.60
YZP-8	12.00	3.80	0.08	45.40	35.30
YZP-9	10.40	4.50	0.05	45.00	31.40
YZP-10	11.50	5.10	0.20	52.80	35.00
Mean value	10.98	3.85	0.09	50.41	38.14

3.3 Content

3.3.1 System suitability. The naringin peak was well separated from adjacent chromatographic peaks (Fig. 4), with resolutions all ≥1.5 and tailing factors in the range of 0.95 – 1.05, indicating good suitability of the HPLC method.



NOTE A: Naringin reference standard; B: Test sample of pomelo peel.

Fig. 4 HPLC chromatograms of the reference standard and the test sample.

3.3.2 Linear relationship. A calibration curve was constructed by plotting the peak areas (y) against seven different concentrations of the reference solution (x, mg/mL). The regression equation for naringin was $y = 16\,659x - 18.456$, with a correlation coefficient (r^2) of 0.999 9 (Fig. 5). The linear range was 0.002 5 – 0.5 mg/mL.

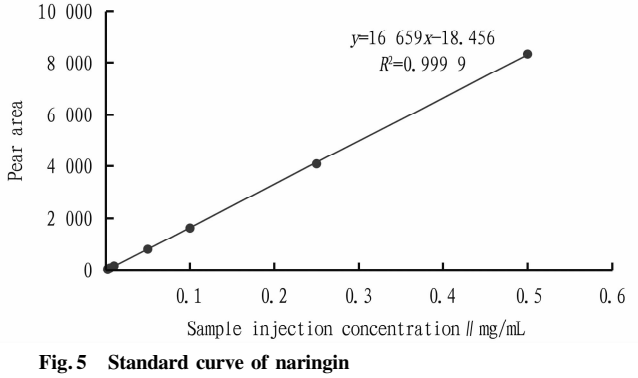


Fig. 5 Standard curve of naringin

3.3.3 Precision. The reference solution was injected six times consecutively within one day, and the peak areas of naringin were measured. The results (Table 3) showed that the *RSD* was 0.93%, which was less than the required 2%, indicating good instrument precision.

Table 3 Precision test results

Test times	Peak area	*RSD*// %
1	876.096	0.93
2	859.015	
3	859.482	
4	855.871	
5	856.155	
6	854.754	

3.3.4 Stability. The test solution (YZP-6) was injected at room temperature at 0, 4, 10, 14, and 24 h, and the peak areas were measured. The stability was evaluated by *RSD*. The results (Table 4) showed an *RSD* of 0.92%, indicating that the test solution was stable within 24 h.

Table 4 Stability test results

Injection time// h	Pear area	Content// %	*RSD*// %
0	1 774.096	2.69	0.924
4	1 776.465	2.69	
10	1 783.124	2.70	
14	1 797.450	2.72	
24	1 813.651	2.75	

3.3.5 Repeatability. Six portions of YZP-6 sample powder were accurately weighed, and the peak areas of naringin were determined (Table 5). The *RSD* was calculated as 1.49%, indicating good repeatability of the method.

Table 5 Repeatability test results

Sample	Peak area	Contnt// %	*RSD*// %
1	1 797.882	2.73	1.49
2	1 866.802	2.83	
3	1 801.411	2.73	
4	1 799.640	2.73	
5	1 817.782	2.76	
6	1 799.270	2.73	

3.3.6 Sample recovery rate. Six portions of YZP-6 sample powder were accurately weighed. Each portion was spiked with an amount of naringin reference standard equal to the naringin content in the sample (100% level) and then analyzed. The results (Table 6) showed an average recovery rate of 96.98% with an *RSD* of 0.53%, indicating good accuracy.

Table 6 Sample recovery rate

No.	Sample weight// g	Content of 1 mL sample// g	Content of naringin added// mg	Measured value// mg	Sample recovery rate// %	Average recovery rate// %	*RSD*// %
1	0.200 1	0.111 4	0.110 0	0.217 8	96.67	96.98	0.53
2	0.200 3	0.112 2	0.110 0	0.218 5	96.70		
3	0.200 3	0.112 1	0.110 0	0.219 5	97.58		
4	0.200 7	0.113 6	0.110 0	0.219 1	95.89		
5	0.200 5	0.112 8	0.110 0	0.217 7	95.38		
6	0.200 7	0.113 7	0.110 0	0.218 4	95.16		

3.3.7 Content analysis. Results (Table 7) showed that the naringin content in the ten collected pomelo peel samples ranged from 1.65% to 7.63%, with an average of 3.57%. The naringin content in pomelo peel varied considerably among different producing areas.

Table 7 Determination results of naringin content in 10 batches of samples

Batch No.	Peak area	Naringin content//%	Mean value//%
YZP-1	1 367.717	2.08	2.07 ±0.01
	1 360.006	2.07	
	1 352.483	2.06	
YZP-2	2 270.040	3.43	3.49 ±0.06
	2 310.622	3.50	
	2 346.039	3.55	
YZP-3	2 099.816	3.18	3.17 ±0.02
	2 079.970	3.15	
	2 093.161	3.17	
YZP-4	5 078.511	7.65	7.63 ±0.03
	5 073.640	7.64	
	5 042.988	7.60	
YZP-5	4 444.037	6.70	6.73 ±0.05
	4 504.738	6.79	
	4 453.148	6.71	
YZP-6	1 777.855	2.70	2.69 ±0.01
	1 760.598	2.67	
	1 777.628	2.70	
YZP-7	2 682.981	4.05	4.02 ±0.03
	2 666.181	4.03	
	2 640.160	3.99	
YZP-8	1 831.565	2.78	2.78 ±0.04
	1 861.673	2.82	
	1 809.207	2.74	
YZP-9	1 604.877	2.44	2.46 ±0.02
	1 631.373	2.48	
	1 634.293	2.48	
YZP-10	1 078.780	1.65	1.65 ±0.01
	1 087.120	1.66	
	1 082.260	1.65	
Overall mean			3.67

4 Discussion

Microscopic examination of the powders from the ten batches of pomelo peel revealed identical microscopic characteristics across all samples. However, marked differences were observed in the number of oil cavity cells and the degree of oil droplet fullness. Notably, samples from certain areas, such as YZP-2, showed abundant oil cavities filled with plump oil droplets, indicating a higher level of volatile oil. This inter-regional variation is likely associated with climatic factors, including sunlight, temperature, and precipitation. Regions characterized by ample sunlight and significant diurnal temperature variation appear to favor the biosynthesis and accumulation of volatile oils, thereby promoting the full development of oil cavities.

During the establishment of the TLC method, several developing solvent systems were investigated. The results showed that when using ethyl acetate-methanol-water (8∶2∶2, *v/v/v*) as the developing solvent, with development carried out at 25 ℃ and visualization performed with 10% sulfuric acid solution, the obtained spots were clear and well separated. Moreover, good reproducibility was achieved under different temperatures (4, 25, and 35 ℃), different relative humidities (30% RH and 80% RH), and on silica gel G TLC plates from different manufacturers.

When establishing the HPLC method, the extraction efficiency was evaluated using different ratios of extraction solvents (water, 30% methanol, 50% methanol, 70% methanol, and 100% methanol), different extraction methods, and different extraction times. The results showed that satisfactory extraction efficiency was achieved with 50 mL of 70% methanol and ultrasonication for 30 min. In addition, different column temperatures and columns from different manufacturers were investigated, and good separation was obtained under all the tested conditions, indicating that the chromatographic method has good reproducibility.

The content determination results revealed notable differences in the naringin content of pomelo peel from different production areas. This phenomenon can be attributed to the combined effects of various environmental and genetic factors. First, the synthesis and accumulation of naringin, a flavonoid secondary metabolite in plants, are often adjusted by temperature, light, and precipitation[6]. Second, cultivation and management practices in different regions[7], such as fertilization strategies, pruning methods, and fruit bagging, may also indirectly affect the distribution of secondary metabolites in the peel by altering the microenvironment. Furthermore, soil characteristics are important contributing factors. A previous study[8] indicated that the top five soil factors influencing naringin content are pH, exchangeable magnesium, available copper, available zinc, and total nitrogen. Finally, harvest time and sample processing methods (*e.g.*, fruit maturity, drying method) should not be overlooked. In summary, the interplay of origin-related environmental, biological, and anthropogenic factors collectively shapes the geographic variation in naringin content.

Based on the results obtained from the ten batches of samples, and with reference to the relevant requirements of the General Chapters of the *Chinese Pharmacopoeia* (2020 Edition) and common practices for crude drug quality control, the moisture content of the ten batches ranged from 9.8% to 12.7%, total ash from 3.1% to 5.1%, acid-insoluble ash from 0.04% to 0.2%, water-soluble extractives from 44.1% to 57.2%, ethanol-soluble extractives from 31.4% to 44.6%, and naringin content from 1.65% to 7.63%.

In summary, this study established a rational, feasible, and reproducible quality control method for pomelo peel. Ten batches of samples from different producing areas were analyzed, and the limits for routine test items were preliminarily determined. In addition, TLC and HPLC methods for the analysis of naringin were

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developed. The method is stable and reliable, and can provide a scientific basis for the quality control and further development and utilization of pomelo peel.

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