

Rapid Determination of Hesperidin in Orange Peel Using Accelerated Solvent Extraction and UHPLC

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Key Words

Pharmacopoeia of the People's Republic of China (PPRC),
Natural Herbal Medicine, Flavonoid, Polyphenolic Bioflavonoid

Introduction

Hesperidin, a polyphenolic bioflavonoid, is the predominant flavonoid in orange peel and other citrus fruits. Figure 1 shows its structure. Hesperidin is an antioxidant that enhances the action of vitamin C to lower cholesterol levels. It is also known to have pharmacological action as an anti-inflammatory, antihistaminic, and antiviral agent.^{1,2} The Pharmacopoeia of the People's Republic of China (PPRC) 2010 recommends its extraction from fruits with a Soxhlet extraction method using ligarine and methanol.³ That method is both time- and solvent-consuming, requiring ≥ 5 h and >200 mL of ligarine and methanol for each sample. The PPRC 2010 also recommends the determination with a 12 min reversed-phase high-performance liquid chromatography (HPLC) method.

Goal

To develop a more efficient and cost-effective method to determine hesperidin in orange peel and other citrus fruits.

Equipment

- Thermo Scientific Dionex UltiMate 3000 rapid separation LC (RSLC) system, including:
 - HPG-3400RS Binary Rapid Separation Pump
 - WPS-3000RS Rapid Separation Autosampler
 - TCC-3000RS Thermostatted Column Compartment
 - DAD-3000RS Rapid Separation Diode Array Detector with Semi-Analytical Flow Cell, SST, 5 μ L Volume, 7 mm Path Length
- Thermo Scientific Dionex Chromeleon Chromatography Data System software version 6.80, SR9, or above
- Thermo Scientific Dionex ASE 350 Accelerated Solvent Extractor

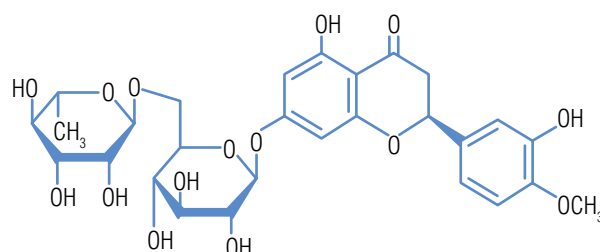


Figure 1. Structure of hesperidin

Sample Extraction

Weigh 1 g of ground orange peel sample and mix it with a suitable amount of diatomaceous earth (DE Dispersant for ASE, P/N 06281). Place the mixture in a separate 10 mL stainless steel extraction cell equipped with two cellulose filters on the bottom. Samples will nearly fill the cells. Extract loaded cells with the Dionex ASE™ 350 system using the optimized conditions shown in Table 2. The use of ligarine to extract naphtha ingredients (step 1) will eliminate their interference for subsequent hesperidin determination. Discard the extract from this step and extract the sample cell again by methanol (step 2). Transfer the methanol extracts into a separate 25 mL volumetric flask and bring to volume with deionized water. Filter the diluted extracts through a 0.4 μ m filter before injection onto the UHPLC system.

Table 1. Comparison of extraction methods using Soxhlet extraction and the Dionex ASE 350

Extraction Method	Soxhlet Extraction	Dionex ASE 350
Sample Amount (g)	1	1
Solvent Amount (mL)	200	40
Time (min)	300	35
Detected Amount of Hesperidin (%)	5.2	6.3
RSD	7.4	3.1

Conclusion

This work demonstrates a quick and efficient method to determine hesperidin in orange peel and other citrus fruits. The Dionex ASE 350 system, an automated extraction system that uses high temperature and pressure to perform quick extractions, was used for the sample extraction. The separation was performed on a Dionex UltiMate™ 3000 RSLC system. The total analysis time—including extraction and separation—for an orange peel sample was ~40 min (35 min for extraction and 4 min for separation), and solvent consumed for each extraction was 40 mL. Table 1 compares the Soxhlet approach with the ASE extractor method; use of the Dionex ASE 350 saves significant time and solvent with similar results. Figure 2 shows the chromatograms of hesperidin in orange peel samples separated on the Thermo Scientific Acclaim RSLC 120 C18 column.

References

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Column: Acclaim™ RSLC 120 C18,
(2.2 μm, 3 × 30 mm, P/N 071605)
Mobile Phase: Methanol/water/acetic acid, 31/65/4 (v/v)
Flow Rate: 0.5 mL/min
Inj. Volume: 1 μL
Temperature: 40 °C
Detection: UV, 283 nm
Chromatograms: a. Mobile phase
b. Hesperidin standard (2 μg/mL)
c. Orange peel sample 1
d. Orange peel sample 2 (100-fold dilution)
e. Lemon peel sample (50-fold dilution)

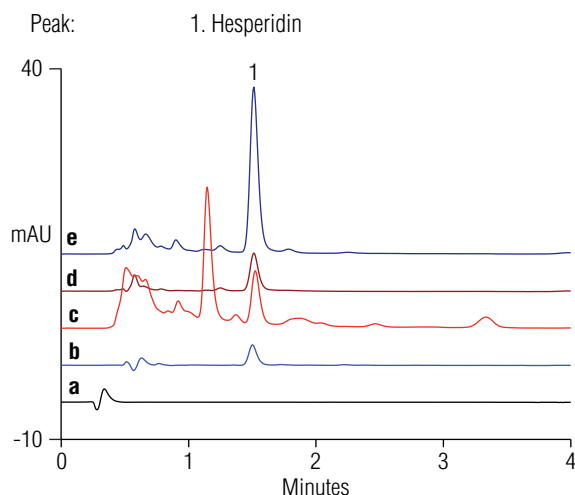


Figure 2. Chromatograms of hesperidin in orange and lemon peel samples

Table 2. Dionex ASE 350 instrument conditions

Conditions	Step 1	Step 2
Solvent	Ligarine	Methanol
Temperature	120 °C	
Heat Time	6 min	
Static Time	3 min	5 min
Static Cycles	2	
Flush	60%	
Purge Time	200 s	90 s

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