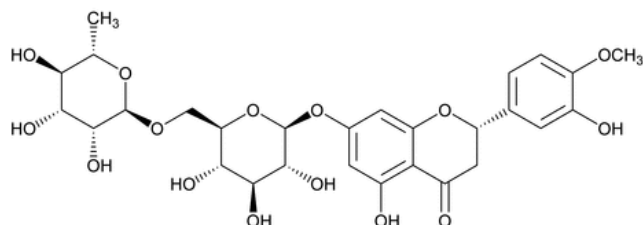


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Hesperidin



$C_{28}H_{34}O_{15}$ 610.57

4H-1-Benzopyran-4-one, 7-[[6-O-(6-deoxy- α -L-mannopyranosyl)- β -D-glucopyranosyl]oxy]-2,3-dihydro-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-, (2S)-;

(2S)-5-Hydroxy-2-(3-hydroxy-4-methoxyphenyl)-4-oxo-3,4-dihydro-2H-chromen-7-yl 6-O-(α -L-rhamnopyranosyl)- β -D-glucopyranoside CAS RN®: 520-26-3.

DEFINITION

Hesperidin contains NLT 90.0% and NMT 102.0% of hesperidin ($C_{28}H_{34}O_{15}$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A.** ▲ [SPECTROSCOPIC IDENTIFICATION TESTS \(197\)](#), *Infrared Spectroscopy: 197K* ▲ (CN 1-MAY-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY

• PROCEDURE

Solution A: Dilute 5 mL of glacial acetic acid with water to 1000 mL.

Mobile phase: Methanol and *Solution A* (30:70)

System suitability solution: 0.2 mg/mL each of [USP Isonaringin RS](#), [USP Hesperidin RS](#), [USP Neohesperidin RS](#), [USP Diosmin RS](#), and [USP Didymnin RS](#) in dimethyl sulfoxide

Standard solution: 1.0 mg/mL of [USP Hesperidin RS](#) in dimethyl sulfoxide

Sample solution: 1.0 mg/mL of Hesperidin in dimethyl sulfoxide

Chromatographic system

(See [Chromatography \(621\)](#), *System Suitability*.)

Mode: LC

Detector: UV 284 nm

Column: 3.9-mm × 15-cm; 4- μ m packing L1

Column temperature: 40°

Flow rate: 1.0 mL/min

Injection volume: 10 μ L

Run time: At least 5 times the retention time of hesperidin

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for hesperidin and its related compounds are shown in [Table 1](#).]

Suitability requirements

Resolution: NLT 1.8 between hesperidin and neohesperidin, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of hesperidin ($C_{28}H_{34}O_{15}$) in the portion of Hesperidin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of [USP Hesperidin RS](#) in the *Standard solution* (mg/mL)

C_U = concentration of Hesperidin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–102.0% on the dried basis

IMPURITIES

• [RESIDUE ON IGNITION \(281\)](#)

Sample: 1.0 g

Acceptance criteria: NMT 0.2%

• RELATED COMPOUNDS

Solution A, Mobile phase, System suitability solution, Chromatographic system, and System suitability: Proceed as directed in the Assay.

Standard solution: 0.05 mg/mL of [USP Hesperidin RS](#) in dimethyl sulfoxide

Sample solution: 1.0 mg/mL of Hesperidin in dimethyl sulfoxide

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Hesperidin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times F \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of hesperidin from the *Standard solution*

C_S = concentration of [USP Hesperidin RS](#) in the *Standard solution* (mg/mL)

C_U = concentration of Hesperidin in the *Sample solution* (mg/mL)

F = correction factor for each individual impurity (see [Table 1](#))

Acceptance criteria: See [Table 1](#). [NOTE—Disregard any impurity less than 0.1%.]

Table 1

Name	Relative Retention Time	Correction Factor (F)	Acceptance Criteria, NMT (%)
Eriocitrin ^a	0.4	1.00	1.0
Isonaringin	0.7	1.07	4.0
Hesperidin	1.0	—	—
Neohesperidin	1.2	0.91	1.0
Diosmin	1.5	1.67	1.0
Naringenin ^b	2.6	0.51	1.0
Didymin	3.0	1.02	



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