Working electrode: Gold
Reference electrode: pH, silver–silver chloride
Electrochemical waveform: See *Table 4*.

Table 4

Time (s)	Potential (V)	Integration
0.00	0.10	
0.20	0.10	Start
0.40	0.10	End
0.41	–2.00	
0.42	–2.00	
0.43	0.60	
0.44	–0.10	
0.50	–0.10	

Column: 4-mm × 25-cm; packing L47
Guard column: 4-mm × 5-cm; packing L47
Column temperature: 30°
Flow rate: 0.4 mL/min
Injection size: 10 µL
System suitability
Sample: *Standard Identification #5*
[NOTE—The relative retention times for inositol, mannose, and glucose are 0.68, 0.95 and 1.0, respectively.]
Suitability requirement:
Resolution: NLT 1.5 between mannose and glucose
Analysis
Samples: *Standard solutions* and *Sample solution*
Calculate the ratios of the peak area response of the glucose and mannose to the peak area response of the internal standard from the *Standard solutions*. Make two standard response lines by plotting the peak area response ratio versus the amount (µg) of the glucose and mannose in the *Standard solutions*. Calculate the ratio of the peak area response of the glucose and mannose to the peak area response of the internal standard from the *Sample solution*. From the calculated ratios of peak responses for glucose and mannose and their respective standard response lines, determine the content of glucose, C_G, and mannose, C_M, both in µg, in the *Sample solution*.
Calculate the percentage of mannose in the portion of Beta Glucan taken:

$$\text{Result} = C_M/(C_M + C_G) \times 100$$

C_M = content of mannose in the *Sample solution* from the mannose regression line (µg)
C_G = content of glucose in the *Sample solution* from the glucose regression line (µg)

Acceptance criteria: NMT 1.0% mannose, as a function of total hexose recovered (glucose and mannose)

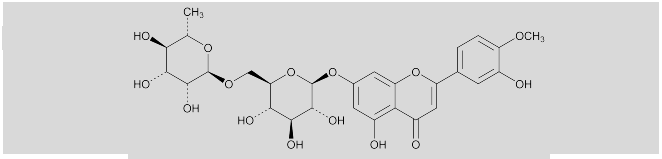
- **RESIDUE ON IGNITION ⟨281⟩:** NMT 2.5%
- **LOSS ON DRYING ⟨731⟩**
Sample: 1 g
Analysis: Dry the *Sample* at 105° for 3 h.
Acceptance criteria: NMT 8.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight and light-resistant containers.
- **USP REFERENCE STANDARDS ⟨11⟩**
USP Beta Glucan RS
USP Dextrose RS
USP Inositol RS
USP Mannose RS■2S *(USP35)*

Add the following:

■Diosmin



$C_{28}H_{32}O_{15}$ 608.54

5-Hydroxy-2-(3-hydroxy-4-methoxyphenyl)-7-[(2*S*,3*R*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-6-[[(2*R*,3*R*,4*R*,5*R*,6*S*)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxymethyl]oxan-2-yl]oxychromen-4-one;
7-[[6-*O*-(6-Deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxy]-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-4*H*-1-benzopyran-4-one [520-27-4].

DEFINITION

Diosmin contains NLT 90.0% and NMT 102.0% of diosmin ($C_{28}H_{32}O_{15}$), calculated on the anhydrous basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION ⟨197K⟩**
- **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**
Mobile phase: Methanol, acetonitrile, acetic acid, and water (27:2:6:65)
Standard solution: 1.0 mg/mL of USP Diosmin RS in dimethyl sulfoxide
System suitability solution: 1 mg/mL of USP Diosmin for System Suitability RS in dimethyl sulfoxide
Sample solution: 1.0 mg/mL of Diosmin in dimethyl sulfoxide
Chromatographic system
(See *Chromatography ⟨621⟩, System Suitability*.)
Mode: LC
Detector: UV 275 nm
Column: 4.6-mm × 10-cm; 3-µm packing L1
Column temperature: 40°
Flow rate: 1.2 mL/min
Injection size: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*.
[NOTE—Allow the run time about 6 times the diosmin retention time. The relative retention times for diosmin, acetoisovanillone, hesperidin, isorhoifolin, linarin, and diosmetin are 1, 0.5, 0.6, 0.8, 2.6, and 4.5, respectively.]
Suitability requirements
Chromatogram similarity: The chromatogram from the *System suitability solution* is similar to the Reference Chromatogram provided with the USP Diosmin for System Suitability RS being used.
Resolution: NLT 2.5 between hesperidin and isorhoifolin, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of diosmin ($C_{28}H_{32}O_{15}$), in the portion of Diosmin 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 Diosmin RS in the *Standard solution* (mg/mL)
C_U = concentration of Diosmin in the *Sample solution* (mg/mL)

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

IMPURITIES

- **RESIDUE ON IGNITION ⟨281⟩**
  Sample: 1.0 g
  Acceptance criteria: NMT 0.2%
- **HEAVY METALS,** *Method II* **⟨231⟩**
  Sample: 2.0 g
  Acceptance criteria: NMT 20 ppm
- **LIMIT OF IODINE**
  Determine the total content of iodine by potentiometry, using an iodide-selective electrode, after oxygen flask combustion (see *Oxygen Flask Combustion* ⟨471⟩).
  Sample solution: [**CAUTION—**Observe rigorously the precautions set forth for *Procedure* under *Oxygen Flask Combustion* ⟨471⟩.] Wrap 0.100 g of Diosmin in a piece of free-halide filter paper, and place it in the platinum gauze specimen holder. Introduce into the flask 50.0 mL of a 0.2 g/L solution of hydrazine. Flush the flask with oxygen for 10 min. Ignite the filter paper. Stir the contents of the flask immediately after the end of the combustion to dissolve completely the combustion products. Continue stirring for 1 h.
  Standard solution: 33.2 µg/mL of potassium iodide in water, equivalent to 25.4 µg/mL of iodine
  Potassium nitrate solution: 200 mg/mL of potassium nitrate in 0.1 M nitric acid
  Analysis
  Samples: *Sample solution* and *Standard solution*
  Transfer 30 mL of *Potassium nitrate solution* to a beaker, immerse the electrodes, and stir for 10 min. The potential (nU_1) must remain stable. Measure the potential (nU_1). Add 1 mL of the *Sample solution*, and measure the potential (nU_2).
  Transfer 30 mL of *Potassium nitrate solution* to a beaker, immerse the electrodes, and stir for 10 min. The potential (nS_1) must remain stable. Measure the potential (nS_1). Add 80 µL of the *Standard solution*, and measure the potential (nS_2).
  Acceptance criteria: NMT 0.1%: The absolute value $|(nU_2) - (nU_1)|$ is not higher than the absolute value $|(nS_2) - (nS_1)|$.
- **RELATED COMPOUNDS**
  Mobile phase, System suitability solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
  Standard solution: 0.05 mg/mL of USP Diosmin RS in dimethyl sulfoxide
  System suitability
  Sample: *System suitability solution*. [NOTE—Allow the run time about 6 times that of the diosmin retention time. The relative retention times for diosmin, acetoisovanillone, hesperidin, isorhoifolin, linarin, and diosmetin are 1, 0.5, 0.6, 0.8, 2.6, and 4.5, respectively.]
  System suitability requirements
  Chromatogram similarity: The chromatogram from the *System suitability solution* is similar to the Reference Chromatogram provided with the USP Diosmin for System Suitability RS being used.
  Resolution: NLT 2.5 between hesperidin and isorhoifolin, *System suitability solution*
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of each impurity in the portion of Diosmin taken: [NOTE—Disregard any impurity less than 0.1%.]

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

r_U = peak response for each impurity from the *Sample solution*
r_S = peak response for diosmin from the *Standard solution*
C_S = concentration of USP Diosmin RS in the *Standard solution* (mg/mL)
C_U = concentration of Diosmin in the *Sample solution* (mg/mL)
F = correction factor for each individual impurity (see *Table 1*)

Acceptance criteria
Total impurities: NMT 10%
Individual impurities: See *Table 1*.
Total other impurities and acetoisovanillone: NMT 1%

Table 1

Name	Relative Retention Time	Correction Factor (*F*)	Acceptance Criteria, NMT (%)
Acetoisovanillone[a]	0.5	0.3	1
Hesperidin[b]	0.6	1	5
Isorhoifolin[c]	0.8	1	3
Linarin[d]	2.6	1	3
Diosmetin[e]	4.5	0.5	3
Any other impurity	—	1	1
Total impurities	—	—	10

[a] 1-(3-Hydroxy-4-methoxyphenyl)ethanone.
[b] (2S)-7-[[6-*O*-(6-Deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxy]-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-2,3-dihydro-4*H*-1-benzopyran-4-one.
[c] 7-[[6-*O*-(6-Deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxy]-5-hydroxy-2-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one.
[d] 7-[[6-*O*-(6-Deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxy]-5-hydroxy-2-(4-methoxyphenyl)-4*H*-1-benzopyran-4-one.
[e] 5,7-Dihydroxy-2-(3-hydroxy-4-methoxyphenyl)-4*H*-1-benzopyran-4-one.

SPECIFIC TESTS

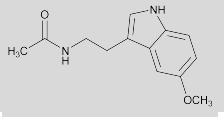
- **WATER DETERMINATION,** *Method Ia* **⟨921⟩**
  Sample: 0.3 g
  Acceptance criteria: NMT 6.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed, tight containers.
- **USP REFERENCE STANDARDS ⟨11⟩**
  USP Diosmin RS
  USP Diosmin for System Suitability RS■2S *(USP35)*

Add the following:

■Melatonin



$C_{13}H_{16}N_2O_2$ 232.28
N-Acetyl-5-methoxytryptamine;
N-(2-(5-Methoxy-1*H*-indol-3-yl)ethyl) acetamide [73-31-4].

DEFINITION

Melatonin contains NLT 98.5% and NMT 101.5% of melatonin ($C_{13}H_{16}N_2O_2$), calculated on the dried basis.