Edition: BP 2025 (Ph. Eur. 11.6 update)

Melatonin Capsules

General Notices

NOTE: *This monograph has been developed to cover unlicensed formulations.*

Action and use

Treatment of sleep onset insomnia.

DEFINITION

Melatonin Capsules contain Melatonin.

The capsules comply with the requirements stated under Capsules and with the following requirements. Where appropriate, the capsules also comply with the requirements stated under Unlicensed Medicines.

Content of melatonin, $C_{13}H_{16}N_2O_2$

90.0 to 105.0% of the stated amount.

IDENTIFICATION

A. The *light absorption*, Appendix II B, of solution (1) obtained in the test for Dissolution, in the range 200 to 350 nm, exhibits a maximum at 278 nm.
B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.
(b) Use 900 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 45 minutes withdraw a 10-mL sample of the medium, filter and dilute the filtered solution, if necessary, with sufficient 0.1M *hydrochloric acid* to produce a solution expected to contain about 0.0001% w/v of Melatonin. Measure the *absorbance* of the filtered sample at the maximum at 278 nm, Appendix II B, using 0.1M *hydrochloric acid* in the reference cell.
(2) Measure the *absorbance* of a 0.0001% w/v solution of *melatonin BPCRS* in 0.1M *hydrochloric acid* using 0.1M *hydrochloric acid* in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of melatonin, $C_{13}H_{16}N_2O_2$, in the medium from the absorbances obtained and using the declared content of $C_{13}H_{16}N_2O_2$ in *melatonin BPCRS*.

LIMITS

The amount of melatonin released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a mixture of 20 volumes of *acetonitrile* and 80 volumes of a 0.245% w/v solution of *potassium dihydrogen orthophosphate*, previously adjusted to pH 3.0 with 20% v/v *orthophosphoric acid*.

(1) To a quantity of the contents of the capsules containing 25 mg of Melatonin add 15 mL, mix with the aid of ultrasound for 10 minutes and then shake vigorously for 60 minutes. Dilute to 25 mL, mix well, filter and dilute 1 volume of the filtrate to 2 volumes.
(2) 0.0005% w/v of *melatonin BPCRS*.
(3) Dilute a mixture of 10 volumes of solution (2) and 1 volume of a 0.025% w/v solution of *5-methoxytryptamine BPCRS* to 100 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Zorbax Agilent Eclipse XDB is suitable).
(b) Use gradient elution and the mobile phase described below.
(c) Use a flow rate of 0.25 mL per minute.
(d) Use a column temperature of 40°.
(e) Use a detection wavelength of 278 nm.
(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 0.245% w/v of *potassium dihydrogen orthophosphate*, adjusted to pH 3.0 with 20% v/v *orthophosphoric acid*.

Mobile phase B *acetonitrile*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-20	90→70	10→30	linear gradient
20-36	70→30	30→70	linear gradient
36-40	30→90	70→10	linear gradient
40-45	90	10	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the retention time of melatonin is about 37.8 minutes and the retention of 5-methoxytryptamine relative to that of melatonin is about 0.63.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to melatonin and 5-methoxytryptamine is at least 12.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak due to 5-methoxytryptamine is not greater than the area of the peak due to 5-methoxytryptamine in the chromatogram obtained with solution (3) (0.5%);

the area of any other *secondary peak* is not greater than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%);

the sum of total impurities is not more than 1.0%.

Disregard any peak with an area less than 0.5 times the area of the peak due to melatonin in the chromatogram obtained with solution (3) (0.05%).

Uniformity of content

Capsules containing less than 2 mg and/or less than 2% w/w of Melatonin comply with the requirements stated under Capsules using the following method of analysis.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 20 mL of *methanol (30%)* to the contents and shell of one capsule, mix with the aid of ultrasound for 10 minutes and then shake for 60 minutes. Dilute 1 volume of the resulting solution to 2 volumes with *methanol (30%)*. Dilute the resulting solution, if necessary, with sufficient *methanol (30%)* to produce a solution containing 0.0025% w/v of Melatonin.
(2) Shake 25 mg of *melatonin BPCRS* with 15 mL of *methanol (30%)* for 10 minutes and add sufficient *methanol (30%)* to produce 25 mL. Dilute 1 volume of the resulting solution to 10 volumes with *methanol (30%)* and further dilute 1 volume to 4 volumes with *methanol (30%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 3 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (4 µm) (Phenomenex Synergi-Hydro RP is suitable).
(b) Use isocratic elution and the mobile phase described below.
(c) Use a flow rate of 1 mL per minute.
(d) Use a column temperature of 40°.
(e) Use a detection wavelength of 285 nm.
(f) Inject 20 µL of each solution.

MOBILE PHASE

400 volumes of *methanol* and 600 volumes of *water* containing 1 volume of *orthophosphoric acid*.

DETERMINATION OF CONTENT

Calculate the content of $C_{13}H_{16}N_2O_2$ in each capsule using the declared content of $C_{13}H_{16}N_2O_2$ in *melatonin BPCRS*.

ASSAY

For capsules containing less than 2 mg and/or less than 2% w/w of Melatonin

Use the average of the individual results obtained in the test for Uniformity of content.

For capsules containing 2 mg or more and 2% w/w or more of Melatonin

Weigh the contents of 20 capsules. Mix and powder if necessary. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) To a quantity of the mixed capsule contents containing 20 mg of Melatonin add 20 mL of *methanol (30%)*, mix with the aid of ultrasound for 10 minutes and then shake for 60 minutes. Dilute 1 volume of the resulting solution to 10 volumes with *methanol (30%)*.
(2) Shake 25 mg of *melatonin BPCRS* with 15 mL of *methanol (30%)* for 10 minutes; add sufficient *methanol (30%)* to produce 25 mL and dilute 1 volume to 10 volumes with *methanol (30%)*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Uniformity of content may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{13}H_{16}N_2O_2$ in the capsules using the declared content of $C_{13}H_{16}N_2O_2$ in *melatonin BPCRS*.

STORAGE

Melatonin Capsules should be protected from light.

IMPURITIES

The impurities limited by the requirements of this monograph include 5-methoxytryptamine.