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Method of analysis for Co-enzyme Q10

$C_{59}H_{90}O_4$ 863.37

2,5-Cyclohexadiene-1,4-dione, 2-[(2*E*,6 *E*,10 *E*,14 *E*,18 *E*,22 *E*,26 *E*,30 *E*,34 *E*)-3,7,11,15,19,23,27,31,35,39-decamethyl-2,6,10,14,18,22,26,30,34,38-tetracontadecaenyl]-5,6- dimethoxy-3-methyl.

2-[(*all-E*)-3,7,11,15,19,23,27,31,35,39-Decamethyl-2,6,10,14,18,22,26,30,34,38-tetracontadecaenyl)-5,6-dimethoxy-3-methyl- *p*-benzoquinone [*303-98-0*].

» Ubidecarenone (Coenzyme Q_{10}) contains not less than 98.0 percent and not more than 101.0 percent of $C_{59}H_{90}O_4$, calculated on the anhydrous basis.

Packaging and storage— Preserve in well-closed, light-resistant containers.

USP Reference standards 〈 11 〉— *USP Ubidecarenone RS* . *USPUbidecarenone for System Suitability RS*.

Identification—

A: *Infrared Absorption* 〈 197K 〉.

B: Dissolve about 50 mg of Ubidecarenone in 1 mL of ethyl ether, and add 10 mL of dehydrated alcohol. To 2 mL of this solution, add 3 mL of dehydrated alcohol and 2 mL of dimethyl malonate, add 1 mL of potassium hydroxide solution (1 in 5) dropwise, and mix: a blue color appears.

Water, *Method I* 〈 921 〉: not more than 0.2%.

Residue on ignition 〈 281 〉: not more than 0.1%.

Heavy metals, *Method II* 〈 231 〉: 0.002%.

Chromatographic purity—

TEST 1: COENZYMES Q_7, Q_8, Q_9, Q_{11} AND RELATED IMPURITIES—

Mobile phase— Proceed as directed in the *Assay.*

Chromatographic system— Proceed as directed in the *Assay.* To evaluate the system suitability requirements, use the *System suitability preparation*, as prepared in the *Assay.*

Standard solution and *Test solution*— Use the *Standard preparation* and the *Assay preparation*, as prepared in the *Assay.*

Procedure— Proceed as directed in the *Assay*, measure all the peak areas, and calculate the percentage of impurities in the portion of Ubidecarenone taken by the formula:

$$100(r_i / r_s),$$

in which r_i is the sum of all peak responses, other than that for ubidecarenone, obtained from the *Test solution;* and r_s is the sum of all peak responses. Not more than 1.0% is found.

TEST 2: UBIDECARENONE *(2 Z)*- ISOMER AND RELATED IMPURITIES—

Mobile phase— Prepare a filtered and degassed mixture of *n*-hexane and ethyl acetate (97:3).

System suitability solution— Prepare a solution of *USP Ubidecarenone for System Suitability RS* in *n*-hexane having a concentration of about 1 mg per mL.

Test solution— Prepare a solution of Ubidecarenone in *n*-hexane having a concentration of about 1 mg per mL.

Chromatographic system— The liquid chromatograph is equipped with a 275-nm detector and a 4.6-mm × 25-cm column that contains packing L3. The flow rate is about 2.0 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure:* the relative retention times are about 0.85 for ubidecarenone (2 *Z*)-isomer and 1.0 for ubidecarenone; and the resolution, *R*, between ubidecarenone (2 *Z*)-isomer and ubidecarenone is not less than 1.5.

Procedure—Inject a volume of the *Test solution* (about 20 µL) into the chromatograph, record the chromatogram, and measure all the peak responses. Calculate the percentage of impurities in the portion of Ubidecarenone taken by the formula:

$$100(r_i / r_s),$$

in which r_i is the sum of all peak responses, other than that for ubidecarenone; and r_s is the sum of all peak responses. Not more than 1.0% is found.

Calculate the percentage of total impurities as the sum of the percentages obtained by *Test 1* and *Test 2:* not more than 1.5% of total impurities is found.

Assay—

Mobile phase— Prepare a filtered and degassed mixture of methanol and dehydrated alcohol (13:7). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨ 621 ⟩).

System suitability preparation— Dissolve accurately weighed quantities of *USP*

Ubidecarenone RS and coenzyme Q_9 in dehydrated alcohol, heating at about 50° for 2 minutes if necessary, to obtain a solution having known concentrations of about 0.5 mg of each per mL.

Standard preparation— Dissolve an accurately weighed quantity of *USP Ubidecarenone RS* in dehydrated alcohol, heating at about 50° for 2 minutes if necessary, to obtain a solution having a known concentration of about 1.0 mg per mL.

Assay preparation— Transfer about 50 mg of Ubidecarenone, accurately weighed, to a 50-mL volumetric flask, dissolve in dehydrated alcohol, heating at about 50° for 2 minutes if necessary, cool, dilute with dehydrated alcohol to volume, and mix.

Chromatographic system (see *Chromatography* 〈 621 〉) — The liquid chromatograph is equipped with a 275-nm detector and a 5-mm × 15-cm column that contains packing L1, and is maintained at a temperature of 35°. The flow rate is adjusted to obtain a retention time of about 11 minutes. Chromatograph the *System suitability preparation*, and record the peak responses as directed for *Procedure:* the relative retention times are about 0.75 for coenzyme Q_9 and 1.0 for ubidecarenone; the resolution, *R*, between coenzyme Q_9 and ubidecarenone is not less than 4; and the relative standard deviation for replicate injections is not more than 0.8%.

Procedure— Separately inject equal volumes (about 5 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{59}H_{90}O_4$ in the portion of Ubidecarenone taken by the formula:

$$50C(r_U / r_S),$$

in which *C* is the concentration, in mg per mL, of *USP Ubidecarenone RS* in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.