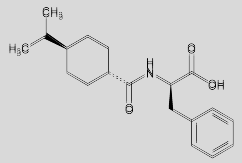
***Add the following:***

## ▲Nateglinide



$C_{19}H_{27}NO_3$ 317.42

D-Phenylalanine, *N*-[[*trans*-4-(1-methylethyl)cyclohexyl] carbonyl]-;
(–)-*N*-[(*trans*-4-Isopropylcyclohexyl)carbonyl-D-phenylalanine [105816-04-4].

**DEFINITION**

Nateglinide contains NLT 98.0% and NMT 102.0% of $C_{19}H_{27}NO_3$, calculated on the dried 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**

**Buffer:** 8.5 g/L of anhydrous dibasic sodium phosphate in water. Adjust with phosphoric acid to a pH of 7.5.
**Mobile phase:** Methanol and *Buffer* (1:1)
**Standard solution:** 1.0 mg/mL of nateglinide prepared as follows: transfer USP Nateglinide RS to a suitable volumetric flask, dissolve first in methanol, using 50% of the final volume, and then dilute with *Buffer* to volume.
**System suitability stock solution:** 0.2 mg/mL each of USP Nateglinide Related Compound C RS and DL-phenylalanine in methanol. [NOTE—Sonicate, if necessary.]
**System suitability solution:** Transfer USP Nateglinide RS to a suitable volumetric flask, dissolve first in methanol, using 45% of the final volume, add *System suitability stock solution* equal to 5% of the final volume, and then dilute with *Buffer* to volume to obtain a solution containing about 1.0 mg/mL of nateglinide and about 0.01 mg/mL each of nateglinide related compound C and DL-phenylalanine.
**Sample solution:** Transfer about 100 mg of Nateglinide to a 100-mL volumetric flask, dissolve in 50 mL of methanol, and dilute with *Buffer* to volume.
**Chromatographic system**
(See *Chromatography* ⟨621⟩, *System Suitability*.)
**Mode:** LC
**Detector:** UV 210 nm
**Column:** 6-mm × 15-cm; 6-µm packing L71 (see *Chromatographic Reagents* under *Reagents, Indicators, and Solutions*)
**Column temperature:** 30°
**Flow rate:** 1 mL/min
**Injection size:** 20 µL
**System suitability**
**Samples:** *Standard solution* and *System suitability solution*
**Suitability requirements**
**Resolution:** NLT 0.9 between nateglinide related compound C and nateglinide, *System suitability solution*
**Relative standard deviation:** NMT 1.0%, *Standard solution*
**Analysis**
**Samples:** *Standard solution* and *Sample solution*
Calculate the percentage of nateglinide ($C_{19}H_{27}NO_3$) in the portion of Nateglinide 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 Nateglinide RS in the *Standard solution* (mg/mL)
$C_U$ = concentration of Nateglinide in the *Sample solution* (mg/mL)
**Acceptance criteria:** 98.0%–102.0% on the dried basis

**IMPURITIES**

- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.1%
- **HEAVY METALS,** *Method II* **⟨231⟩:** NMT 10 ppm

***Change to read:***

- **LIMIT OF NATEGLINIDE RELATED COMPOUND A AND OTHER IMPURITIES**

**Buffer:** 7.8 g/L of monobasic sodium phosphate in water. Adjust with phosphoric acid to a pH of 2.5.
**Mobile phase:** Acetonitrile and *Buffer* (7:13)
**Standard solution:** Dissolve USP Nateglinide RS in acetonitrile to obtain a solution having a known concentration of about 0.3 mg/mL. Further dilute this solution with *Mobile phase* to obtain a solution having a known concentration of about 0.06 mg/mL.
**System suitability stock solution:** Dissolve USP Nateglinide Related Compound A RS in acetonitrile to obtain a solution containing about 0.6 mg/mL. Further dilute this solution with *Mobile phase* to obtain a solution containing about 0.12 mg/mL.
**System suitability solution:** Transfer an amount of USP Nateglinide RS to a suitable volumetric flask, dissolve first in acetonitrile using 10% of the final volume, then add *System suitability stock solution* equal to 10% of the final volume, and dilute with *Mobile phase* to volume to obtain a solution containing about 6 mg/mL of nateglinide and about 0.012 mg/mL of nateglinide related compound A.
**Sample solution:** Transfer 60 mg of Nateglinide to a 10-mL volumetric flask, dissolve •in a minimal amount of• (RB1-Oct-2010) acetonitrile, and dilute with *Mobile phase* to volume.
**Chromatographic system**
(See *Chromatography* ⟨621⟩, *System Suitability*.)
**Mode:** LC
**Detector:** UV 210 nm
**Column:** 3.9-mm × 5-cm; 5-µm, packing L7
**Column temperature:** 40°
**Flow rate:** 2 mL/min
**Injection size:** 100 µL
**Run time:** 5 times the retention time of nateglinide
**System suitability**
**Samples:** *Standard solution* and *System suitability solution*
**Suitability requirements**
**Resolution:** NLT 2.5 between nateglinide related compound A and nateglinide, *System suitability solution*
**Relative standard deviation:** NMT 2.0%, *Standard solution*
**Analysis**
**Samples:** *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Nateglinide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$ = peak response of each impurity from the *Sample solution*
$r_S$ = peak response of nateglinide from the *Standard solution*
$C_S$ = concentration of USP Nateglinide RS in the *Standard solution* (mg/mL)
$C_U$ = concentration of Nateglinide in the *Sample solution* (mg/mL)
$F$ = relative response factor (see *Table 1*)

**Acceptance criteria**

**Individual impurities:** See *Table 1*.

**Table 1**

| Name | Relative Retention Time | Relative Response Factor | Acceptance Criteria, NMT (%) |
|---|---|---|---|
| Nateglinide related compound A[a] | 0.5 | 0.015 | 0.2 |
| Ethyl analog[b] | 0.6 | 1.0 | 0.1 |
| Nateglinide | 1.0 | — | — |
| IPP impurity[c] | 3.1 | 1.0 | 0.1 |
| Ester impurity[d] | 4.1 | 0.94 | 0.1 |
| Any other individual impurity | — | 1.0 | 0.1 |

[a] *trans*-4-Isopropylcyclohexylcarboxylic acid.
[b] *N*-(*trans*-4-Ethylcyclohexylcarbonyl)-D-phenylalanine.
[c] *N*-(*trans*-4-Isopropylcyclohexylcarbonyl)-D-phenylalanine-D-phenylalanine.
[d] *N*-(*trans*-4-isopropylcyclohexylcarbonyl)-D-phenylalanine-ethyl ester.

• **LIMIT OF NATEGLINIDE RELATED COMPOUND B**

**Mobile phase:** 0.77 g/L of ammonium acetate in methanol [NOTE—The following solutions are stable for up to 48 h when stored in a refrigerator.]

**Standard solution:** 0.02 mg/mL of USP Nateglinide Related Compound B RS in methanol. [NOTE—Nateglinide related compound B is *N*-(*trans*-4-isopropyl-cyclohexylcarbonyl)-L-phenylalanine.]

**System suitability solution:** 10 mg/mL of USP Nateglinide RS and 0.02 mg/mL of USP Nateglinide Related Compound B RS in methanol

**Sample solution:** 10 mg/mL of Nateglinide in methanol

**Chromatographic system**

(See *Chromatography* ⟨621⟩, *System Suitability*.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 4-mm × 25-cm or 4.6-mm × 25-cm; 5-µm packing L72 (see *Chromatographic Reagents* under *Reagents, Indicators, and Solutions*)

**Column temperature:** 40°

**Flow rate:** 0.8 mL/min. [NOTE—The flow rate can be adjusted as needed to achieve a recommended retention time of nateglinide related compound B at about 25 min.]

**Injection size:** 10 µL

**System suitability**

[NOTE—The elution order is nateglinide related compound B, followed by the nateglinide peak.]

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

**Suitability requirements**

**Resolution:** NLT 0.8 between nateglinide related compound B and nateglinide, *System suitability solution*

**Relative standard deviation:** NMT 5%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of nateglinide related compound B in the portion of Nateglinide taken:

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

$r_U$ = peak response of nateglinide related compound B from the *Sample solution*
$r_S$ = peak response of nateglinide related compound B from the *Standard solution*
$C_S$ = concentration of USP Nateglinide Related Compound B RS in the *Standard solution* (mg/mL)
$C_U$ = concentration of Nateglinide in the *Sample solution* (mg/mL)

**Acceptance criteria:** NMT 0.2%

• **LIMIT OF NATEGLINIDE RELATED COMPOUND C AND PHENYLALANINE**

**Mobile phase, Standard solution, System suitability solution, Sample solution,** and **Chromatographic system:** Proceed as directed in the *Assay*.

**Diluted standard solution:** Dilute the *Standard solution* with *Mobile phase* to obtain a solution having a known concentration of about 0.01 mg/mL of nateglinide.

**Analysis**

**Samples:** *Sample solution* and *Diluted standard solution*

Calculate the percentage of each specified impurity listed in *Table 2* in the portion of Nateglinide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$ = peak response of each impurity from the *Sample solution*
$r_S$ = peak response of nateglinide from the *Diluted standard solution*
$C_S$ = concentration of nateglinide in the *Diluted standard solution* (mg/mL)
$C_U$ = concentration of Nateglinide in the *Sample solution* (mg/mL)
$F$ = relative response factor of each individual impurity (see *Table 2*)

**Acceptance criteria**

**Individual impurities:** See *Table 2*.

**Table 2**

| Name | Relative Retention Time | Relative Response Factor | Acceptance Criteria, NMT (%) |
|---|---|---|---|
| Phenylalanine | 0.2 | 1.5 | 0.2 |
| Nateglinide *cis*-isomer[a] (related compound C) | 0.9 | 0.97 | 0.2 |
| Nateglinide | 1.0 | — | — |

[a] *N*-(*cis*-4-isopropylcyclohexylcarbonyl)-D-phenylalanine.

**Total impurities:** The sum of all impurities found in the tests for *Limit of Nateglinide Related Compound A and Other Impurities*, *Limit of Nateglinide Related Compound B*, and *Limit of Nateglinide Related Compound C and Phenylalanine* is NMT 0.5%.

## SPECIFIC TESTS

***Change to read:***

• **LOSS ON DRYING** ⟨**731**⟩**:** Dry a sample at 105° for 2 h: it loses •NMT 0.5%• (RB 1-Oct-2010) of its weight.

## ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers. Store at room temperature.

• **USP REFERENCE STANDARDS** ⟨**11**⟩
USP Nateglinide RS
(–)-*N*-[(*trans*-4-Isopropylcyclohexyl)carbonyl-D-phenylalanine
$C_{19}H_{27}NO_3$ 317.42
USP Nateglinide Related Compound A RS
*trans*-4-Isopropylcyclohexylcarboxylic acid.
$C_{10}H_{18}O_2$ 170.2
USP Nateglinide Related Compound B RS
*N*-(*trans*-4-Isopropylcyclohexylcarbonyl)-L-phenylalanine.
$C_{19}H_{27}NO_3$ 317.4
USP Nateglinide Related Compound C RS
Nateglinide *cis*-isomer, *N*-(*cis*-4-isopropylcyclohexylcarbonyl)-D-phenylalanine.
$C_{19}H_{27}NO_3$ 317.4▲*USP33*