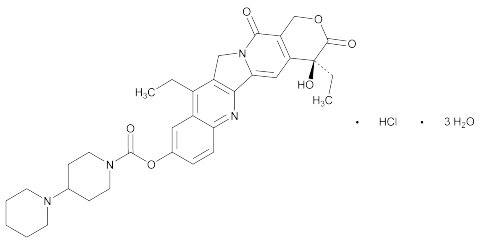
Add the following:

•Irinotecan Hydrochloride



$C_{33}H_{38}N_4O_6 \cdot HCl \cdot 3H_2O$
Anhydrous: 623.14
Trihydrate: 677.18

[1,4′-Bipiperidine]-1′-carboxylic acid, 4,11-diethyl-3,4,12,14-tetrahydro-4-hydroxy-3,14-dioxo-1*H*-pyrano[3′,4′: 6,7]indolizino [1,2-*b*]quinolin-9-yl ester, monohydrochloride, trihydrate, (*S*)-.
(+)-7-Ethyl-10-hydroxycamptothecine 10-[1,4′-bipiperidine]-1′-carboxylate, monohydrochloride, trihydrate [*136572-09-3*].

» Irinotecan Hydrochloride contains not less than 98.0 percent and not more than 102.0 percent of $C_{33}H_{38}N_4O_6 \cdot HCl$, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight, light-resistant containers, and store at controlled room temperature.

USP Reference standards ⟨11⟩**—***USP Irinotecan Hydrochloride RS*. *USP Irinotecan Related Compound B RS*. *USP Irinotecan Related Compound C RS*. *USP Irinotecan Related Compound D RS*.

Identification—

A: *Infrared Absorption* ⟨197K⟩.

B: The retention time of the major peak in the chromatogram of the *Test solution* corresponds to the irinotecan (*S*-enantiomer) peak in the *Identification solution*, as obtained in the test for *Limit of irinotecan hydrochloride enantiomer*.

C: *Chloride* ⟨191⟩—A solution of about 2 mg per mL meets the requirements of the tests for *Chloride* ⟨191⟩.

Microbial enumeration tests ⟨61⟩ and **Tests for specified microorganisms** ⟨62⟩**:** The total aerobic microbial count does not exceed 1000 cfu per g, and the total combined molds and yeasts count does not exceed 100 cfu per g.

Water, *Method 1* ⟨921⟩**:** between 7.0% and 9.0%.

Residue on ignition ⟨281⟩**:** not more than 0.1%.

Heavy metals, *Method II* ⟨231⟩: not more than 10 ppm.

Limit of irinotecan hydrochloride enantiomer—

Mobile phase—Prepare a mixture of hexane, alcohol, and diethylamine (250 : 250 : 1).

Diluent—Prepare a mixture of alcohol and diethylamine (250 : 1).

Resolution solution—Dissolve an accurately weighed quantity of USP Irinotecan Hydrochloride RS and USP Related Compound D RS in *Diluent* to obtain a solution having known concentrations of about 0.1 mg each per mL.

Identification solution—Dissolve an accurately weighed quantity of USP Irinotecan Hydrochloride RS in *Diluent* to obtain a solution having a known concentration of about 1 mg per mL. [NOTE—This solution is used for *Identification* test *B*.]

Standard solution—Dissolve an accurately weighed quantity of USP Irinotecan Related Compound D RS in *Diluent*, quantitatively and stepwise if necessary, to obtain a solution having a known concentration of about 0.0015 mg per mL.

Sensitivity solution—Quantitatively dilute the *Standard solution* with *Diluent* to obtain a solution having a known concentration of about 0.0005 mg per mL.

Test solution—Dissolve an accurately weighed quantity of Irinotecan Hydrochloride in *Diluent* to obtain a solution having a known concentration of about 1 mg per mL.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 370-nm detector and a 4.6-mm × 25-cm column that contains 10-µm packing L40. The flow rate is about 1.0 mL per minute. Chromatograph the *Resolution solution*, and record the peak areas as directed for *Procedure*: the relative retention times are about 0.71 for irinotecan related compound D (*R* -enantiomer) and 1.00 for irinotecan (*S*-enantiomer); and the resolution, *R,* between irinotecan related compound D and irinotecan is not less than 2.5. Chromatograph the *Standard solution,* and record the peak areas as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%. Chromatograph the *Sensitivity solution*, and record the peak area as directed for *Procedure*: the irinotecan related compound D peak should be visible.

Procedure—Inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak areas. Calculate the percentage of irinotecan hydrochloride *R*-enantiomer in the portion of Irinotecan Hydrochloride taken by the formula:

$$100(C_S / C_U)(r_U / r_S)$$

in which C_S is the concentration, in mg per mL, of USP Irinotecan Related Compound D RS in the *Standard solution;* C_U is the concentration, in mg per mL, of Irinotecan Hydrochloride in the *Test solution*; and r_U and r_S are the peak areas for irinotecan related compound D obtained from the *Test solution* and the *Standard solution*, respectively: not more than 0.15% of irinotecan hydrochloride enantiomer is found. [NOTE—Irinotecan related compound D is (*R*)-9-[(1,4′-Bipiperidine)-1′-carbonyloxy]-4,11-diethyl-3,4,12,14-tetrahydro-4-hydroxy-3,14-dioxo-1*H*-pyrano[3′,4′:6,7]indolizino[1,2-*b*] quinoline hydrochloride, trihydrate ($C_{33}H_{38}N_4O_6 \cdot HCl \cdot 3H_2O$, 677.18).]

Related compounds—

Mobile phase and *Diluent*—Prepare as directed in the *Assay*.

Standard stock solution—Use the *Standard preparation,* prepared as directed in the *Assay*.

Standard solution—Quantitatively dilute the *Standard solution* with *Diluent*, stepwise if necessary, to obtain a solution having a known concentration of about 0.002 mg per mL.

Sensitivity solution—Quantitatively dilute the *Standard stock solution* with *Diluent*, stepwise if necessary, to obtain a solution having a known concentration of about 0.0005 mg per mL. [NOTE—The irinotecan hydrochloride in this solution is 0.05% relative to the amount of Irinotecan Hydrochloride in the *Test solution*.]

System suitability stock solution—Dissolve suitable quantities of USP Irinotecan Related Compound B RS and USP Irinotecan Related Compound C RS in methanol, quantitatively and stepwise if necessary, to obtain a solution having known concentrations of about 0.01 mg each per mL.

System suitability solution—Dilute the *System suitability stock solution* with *Diluent* to obtain a solution having a known concentration of about 0.001 mg per mL.

Test solution—Use the *Assay preparation*.

Chromatographic system (see *Chromatography* ⟨621⟩)—Prepare as directed in the *Assay*. Chromatograph the *System suitability solution*, and record the peak areas as directed for *Procedure*: the reso-

lution, *R*, between the peaks of irinotecan related compound B and irinotecan related compound C is not less than 1.1. Chromatograph the *Standard solution*, and record the peak areas as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%. Chromatograph the *Sensitivity solution*, and record the peak area as directed for *Procedure*: the signal-to-noise ratio for the irinotecan peak is not less than 10.

Procedure—Inject equal volumes (about 15 µL) of the *Standard solution* and the *Test solution* into a chromatograph, record the chromatograms, and measure the peak areas. Identify the peaks by their relative retention times: about 0.55 for irinotecan related compound B, 0.60 for irinotecan related compound C, and 1.00 for irinotecan. Calculate the percentage of related compound B, related compound C, and any unspecified impurities in the portion of Irinotecan Hydrochloride taken by the formula:

$$100(C_S / C_U)(r_U / r_S)$$

in which C_S is the concentration, in mg per mL, of USP Irinotecan Hydrochloride RS in the *Standard solution*; C_U is the concentration, in mg per mL, of Irinotecan Hydrochloride in the *Test solution*; r_U is the peak area for each impurity obtained from the *Test solution*; and r_S is the peak area for irinotecan obtained from the *Standard solution*: not more than 0.15% of irinotecan related compound B is found; not more than 0.10% of irinotecan related compound C is found; not more than 0.10% of any unspecified impurity is found; and not more than 0.5% of total impurities is found. Disregard any peak with an area less than the area of the irinotecan peak in the chromatogram obtained from the *Sensitivity solution*. [NOTE—Irinotecan related compound B is (*S*)-4,11-Diethyl-4,9-dihydroxy-1*H*-pyrano[3′,4′:6,7]indolizino[1,2-*b*]quinoline-3,14(4*H*,12*H*)-dione ($C_{22}H_{20}N_2O_5$, 392.40). Irinotecan related compound C is (*S*)-9-[(1,4′-Bipiperidine)-1′-carbonyloxy]-4-methyl-11-ethyl-3,4,12,14-tetrahydro-4-hydroxy-3,14-dioxo-1*H*-pyrano[3′,4′:6,7]indolizino[1,2-*b*]quinoline hydrochloride ($C_{32}H_{36}N_4O_6 \cdot HCl$, 609.11).]

Assay—

Phosphate buffer—Dissolve 2.8 g of monobasic sodium phosphate monohydrate and 1.8 g of 1-octanesulfonic acid sodium salt monohydrate in 1 L of water, and filter the solution.

Mobile phase—Prepare a mixture of *Phosphate buffer*, methanol, and acetonitrile (59 : 24 : 17). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Diluent—Use *Mobile phase* adjusted with diluted hydrochloric acid to a pH of 3.65 ± 0.15.

Standard preparation—Dissolve an accurately weighed quantity of USP Irinotecan Hydrochloride RS in *Diluent* to obtain a solution having a known concentration of about 1 mg per mL.

Assay preparation—Dissolve an accurately weighed quantity of Irinotecan Hydrochloride in *Diluent* to obtain a solution having a known concentration of about 1 mg per mL.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 255-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The flow rate is about 1.5 mL per minute. The column temperature is maintained at 40°. Chromatograph the *Standard preparation*, and record the peak areas as directed for *Procedure*: the tailing factor is not more than 1.5; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Inject equal volumes (about 15 µL) of the *Standard preparation* and the *Assay preparation* into a chromatograph, record the chromatograms, and measure the peak areas. Calculate the percentage of $C_{33}H_{38}N_4O_6 \cdot HCl$ in the portion of Irinotecan Hydrochloride taken by the formula:

$$100(C_S / C_U)(r_U / r_S)$$

in which C_S is the concentration, in mg per mL, of Irinotecan Hydrochloride in the *Standard preparation*; C_U is the concentration, in mg per mL, of Irinotecan Hydrochloride in the *Assay preparation*; and r_U and r_S are the peak areas obtained from the *Assay preparation* and the *Standard preparation*, respectively.• (RB 1-May-2010)