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Etoposide Injection

To view the Notice from the Expert Committee that posted in conjunction with this accelerated revision, please click www.uspnf.com/rb-etoposide-inj-20230331.

DEFINITION

Etoposide Injection contains NLT 90.0% and NMT 110.0% of the labeled amount of etoposide ($C_{29}H_{32}O_{13}$) in a sterile solution in a nonaqueous medium intended for dilution with a suitable parenteral vehicle before intravenous infusion.

IDENTIFICATION

• **A.**

Diluent: Chloroform and methanol (9:1)

Standard solution: 0.8 mg/mL of USP Etoposide RS in *Diluent*

Sample solution: Equivalent to 0.8 mg/mL of etoposide in *Diluent* from the Injection

Chromatographic system

(See *Chromatography ⟨621⟩, Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 10 µL

Developing solvent system: Chloroform, acetone, alcohol, and water (80:25:2.5:0.5)

Spray reagent: Add 10 mL of sulfuric acid with cooling and stirring to 70 mL of dehydrated alcohol in a 100-mL volumetric flask. Dilute with dehydrated alcohol to volume, and mix.

Analysis:

Samples: *Standard solution* and *Sample solution*

Allow the chromatogram to develop until the solvent front has moved 17 cm from the origin. Remove the plate, and allow it to air-dry in a fume hood for 5 min. Replace the plate in the tank, and develop again to a distance of 17 cm from the origin. Remove the plate, and air-dry it in a fume hood for about 20 min. Spray the plate with the *Spray reagent*, and heat in a forced-air oven at 120° for about 15 min.

Acceptance criteria: The appearance and R_F value of the principal spot from the *Sample solution* corresponds to that from the *Standard solution*.

• **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: 2.72 g/L of sodium acetate in water. Adjust with glacial acetic acid to a pH of 4.0.

Mobile phase: Acetonitrile and *Buffer* (26:74)

System suitability solution: 0.3 mg/mL of USP Etoposide Resolution Mixture RS in *Mobile phase*

Standard stock solution: 2.0 mg/mL of USP Etoposide RS in acetonitrile

Standard solution: 0.2 mg/mL of USP Etoposide RS in *Mobile phase* from the *Standard stock solution*

Sample solution: Equivalent to 0.2 mg/mL of etoposide in *Mobile phase* from the Injection

Chromatographic system

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

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 30-cm; packing L11

Flow rate: 1 mL/min

Injection volume: 20 µL

Run time: NLT 1.5 times the retention time of etoposide

System suitability

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

Suitability requirements

Resolution: NLT 1.35 between the etoposide and α-etoposide peaks, *System suitability solution*

Relative standard deviation: NMT 2.0% for etoposide, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of etoposide ($C_{29}H_{32}O_{13}$) in the portion of Injection taken:

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

r_U = peak response of etoposide from the *Sample solution*

r_S = peak response of etoposide from the *Standard solution*

C_S = concentration of USP Etoposide RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of etoposide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

• **Organic Impurities**

Buffer: Prepare as directed in the *Assay*.

Solution A: Acetonitrile and *Buffer* (20:80)

Solution B: Acetonitrile and *Buffer* (60:40)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
15	100	0
30	40	60
40	40	60
42	0	100
45	0	100
47	100	0
50	100	0

Diluent: Acetonitrile and 0.02 M sodium acetate previously adjusted with acetic acid to a pH of 4.0 (30:70)

Standard stock solution: 2.0 mg/mL of USP Etoposide RS in *Diluent*

System suitability stock solution: 0.2 mg/mL of *n*-propylparaben in *Diluent*

System suitability solution: Transfer 5.0 mL of the *System suitability stock solution* and 5.0 mL of the *Standard stock solution* to a 50-mL volumetric flask, and dilute with *Diluent* to volume. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, and dilute with *Diluent* to volume.

Standard solution: 10 µg/mL of USP Etoposide RS from the *Standard stock solution* in *Diluent*

Sample solution: Nominally equivalent to 2.0 mg/mL of etoposide in *Diluent*

Chromatographic system

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

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 15-cm; less than 5-µm packing L11

Flow rate: 1.5 mL/min

Injection volume: 25 µL

Run time: NLT 40 min

System suitability

[Note—Run time is 15 min in isocratic conditions.]

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 1.1 between propylparaben and etoposide

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Injection taken:

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

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of etoposide from the *Standard solution*

C_S = concentration of USP Etoposide RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of etoposide in the *Sample solution* (mg/mL)

Acceptance criteria

Total impurities: NMT 3.0%

SPECIFIC TESTS

• **pH** ⟨791⟩

Sample solution: 5.0 mL of Injection in 45 mL of water

Acceptance criteria: 3.0–4.0

• **Alcohol Determination** ⟨611⟩, *Method II* (if present)**:** 90.0%–110.0% of the labeled amount of alcohol (C_2H_5OH), using *n*-propyl alcohol as the internal standard

• **Bacterial Endotoxins Test** ⟨85⟩

Sample solution: Dilute the Injection with sterile water to obtain 0.31 mg/mL of etoposide activity

Acceptance criteria: NMT 2.0 USP Endotoxin Units/mg of etoposide

• **Benzyl Alcohol Content** (if present)

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

Standard solution: Transfer 0.75 mL of freshly distilled benzyl alcohol, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix. Transfer 1.0 mL of this solution to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Analysis

Samples: *Sample solution* and *Standard solution*

Calculate the percentage of the labeled amount of benzyl alcohol in the portion of Injection taken:

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

r_U = peak response of benzyl alcohol from the *Sample solution*

r_S = peak response of benzyl alcohol from the *Standard solution*

C_S = concentration of benzyl alcohol in the *Standard solution* (mg/mL)

C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

• **Other Requirements:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS

Change to read:

• **Packaging and Storage:** Preserve in single-dose or multiple-dose containers▲, preferably▲ (RB 1-Apr-2023) of Type I glass.

• **Labeling:** Label it to indicate that it must be diluted with suitable parenteral vehicle before intravenous infusion.

• **USP Reference Standards** ⟨11⟩

USP Etoposide RS

USP Etoposide Resolution Mixture RS

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
ETOPOSIDE INJECTION	Documentary Standards Support	SM32020 Small Molecules 3

Chromatographic Database Information: Chromatographic Database

Most Recently Appeared In:
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