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Etoposide Injection

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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