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Doxycycline Hyclate Delayed-Release Tablets

DEFINITION

Doxycycline Hyclate Delayed-Release Tablets contain an amount of Doxycycline Hyclate equivalent to NLT 90.0% and NMT 120.0% of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **Procedure**

Protect solutions containing doxycycline from light.

Solution A: Transfer 3.1 g of monobasic potassium phosphate, 0.5 g of edetate disodium, and 0.5 mL of triethylamine to a 1000-mL volumetric flask. Add about 850 mL of water and mix. Dilute with water to volume and adjust with 1 N sodium hydroxide to a pH of 8.5 ± 0.1. Pass through a suitable filter of 0.22-µm pore size.

Solution B: Methanol

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	90	10
2.0	90	10
4.0	60	40
6.0	90	10
9.0	90	10

Diluent: 0.01 N hydrochloric acid

Standard solution: 0.12 mg/mL of USP Doxycycline Hyclate RS in *Diluent*. Sonicate as needed to dissolve.

Sample solution: Nominally 0.1 mg/mL of doxycycline from NLT 20 Tablets prepared as follows. Transfer a suitable portion of finely powdered Tablets to a suitable volumetric flask. Add 80% of the final volume of *Diluent*, sonicate for about 15 min, shake for about 15 min, and dilute with *Diluent* to volume. Pass through a suitable filter of 0.2-µm pore size and use the filtrate for analysis.

Chromatographic system

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

Mode: LC

Detector: UV 270 nm. For *Identification B*, use a diode array detector in the range of 200–400 nm.

Column: 2.1-mm × 5-cm; 1.7-µm packing L7. [Note—A 1.7-µm guard column with packing L7 was used during method validation.]

Column temperature: 60°

Flow rate: 0.6 mL/min

Injection volume: 5 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) in the portion of Tablets taken:

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

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

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

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

P = potency of doxycycline in USP Doxycycline Hyclate RS (µg/mg)

F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–120.0%

PERFORMANCE TESTS

• **Dissolution ⟨711⟩**

Protect solutions containing doxycycline from light.

Test 1: Proceed as directed for *Dissolution ⟨711⟩, Procedure, Apparatus 1 and Apparatus 2, Delayed-Release Dosage Forms, Method B Procedure*.

Acid stage

Medium: 0.06 N hydrochloric acid; 900 mL, degassed with helium

Apparatus 1: 50 rpm

Time: 20 min

Standard solution: 0.128 mg/mL of USP Doxycycline Hyclate RS in *Medium*. Calculate the concentration, C_S, in mg/mL, of doxycycline using the designated potency, in µg/mg, of doxycycline in USP Doxycycline Hyclate RS. [Note—Sonicate if necessary to dissolve.]

Sample solution: Pass portions of the solution under test through a suitable PVDF filter of 0.45-µm pore size.

Detector: UV 346 nm

Cell: 0.1-cm quartz

Blank: *Medium*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

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

L = label claim (mg/Tablet)

V = volume of *Medium*, 900 mL

Tolerances

Level 1 (6 Tablets tested)**:** No individual value is more than 30% of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) dissolved in 20 min.

Level 2 (6 Tablets tested)**:** NMT 2 individual values of the 12 tested are greater than 30% of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) in 20 min.

Buffer stage

Conduct this stage of testing on separate Tablets, selecting those that were not previously subjected to the *Acid stage* testing.

Medium: pH 5.5 neutralized phthalate buffer (see *Reagents, Indicators, and Solutions—Solutions, Buffer Solutions*); 900 mL, degassed

Apparatus 1: 50 rpm

Time: 30 min

Standard solution: 0.128 mg/mL of USP Doxycycline Hyclate RS in *Medium*. Calculate the concentration, C_S, in mg/mL, of doxycycline using the designated potency, in µg/mg, of doxycycline in USP Doxycycline Hyclate RS. [Note—Sonicate if necessary to dissolve.]

Sample solution: Pass portions of the solution under test through a suitable PVDF filter of 0.45-µm pore size.

Analysis: Determine the percentage of doxycycline ($C_{22}H_{24}N_2O_8$) dissolved by the procedure described for the *Acid stage*.

Tolerances: NLT 85% (*Q*) of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*. Proceed as directed for *Dissolution ⟨711⟩, Procedure, Apparatus 1 and Apparatus 2, Delayed-Release Dosage Forms, Method B Procedure*.

Acid stage

Medium, Apparatus 1, Time, Blank, and **Analysis:** Proceed as directed for *Acid stage* in *Test 1*.

Standard solution: (*L*/900) mg/mL of USP Doxycycline Hyclate RS in *Medium*. Calculate the concentration, C_S, in mg/mL, of doxycycline using the designated potency, in µg/mg, of doxycycline in USP Doxycycline Hyclate RS. Sonicate if necessary to dissolve.

Sample solution: Pass portions of the solution under test through a suitable filter.

Detector: UV 345 nm

Cell: See *Table 2*.

Table 2

Tablet Strength (mg/Tablet)	Cell Size (cm)
75	0.5
100	0.5
150	0.2

Tolerances

Level 1 (6 Tablets tested)**:** No individual value is more than 50% of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) dissolved in 20 min.

Level 2 (6 Tablets tested)**:** NMT 2 individual values of the 12 tested are greater than 50% of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) in 20 min.

Buffer stage

Conduct this stage of testing on separate Tablets, selecting those that were not previously subjected to the *Acid stage* testing.

Medium: pH 5.5 neutralized phthalate buffer (see *Reagents, Indicators, and Solutions—Solutions, Buffer Solutions*); 1000 mL, degassed

Apparatus 1 and **Analysis:** Proceed as directed for *Buffer stage* in *Test 1*.

Time: 45 min

Standard solution: (*L*/1000) mg/mL of USP Doxycycline Hyclate RS in *Medium*. Calculate the concentration, C_S, in mg/mL, of doxycycline using the designated potency, in µg/mg, of doxycycline in USP Doxycycline Hyclate RS. Sonicate if necessary to dissolve.

Sample solution: Pass portions of the solution under test through a suitable filter.

Detector and **Cell:** Proceed as directed for *Acid stage* in *Test 2*.

Tolerances: NLT 70% (*Q*) of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) is dissolved.

Test 3: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 3*. Proceed as directed for *Dissolution ⟨711⟩, Procedure, Apparatus 1 and Apparatus 2, Delayed-Release Dosage Forms, Method B Procedure*.

Acid stage

Apparatus 1 and **Time:** Proceed as directed for *Acid stage* in *Test 1*.

Medium: 0.06 N hydrochloric acid; 900 mL

Standard solution: Prepare the solutions from USP Doxycycline Hyclate RS in *Medium* as directed in *Table 3*. Calculate the concentration, C_S, in mg/mL, of doxycycline using the designated potency, in µg/mg, of doxycycline in USP Doxycycline Hyclate RS.

Table 3

Tablet Strength (mg/Tablet)	Concentration of Doxycycline (mg/mL)
75	0.1
100	0.1
150	0.17

Sample solution: Pass portions of the solution under test through a suitable filter.

Detector: UV 345 nm

Cell: 0.2 cm

Blank: *Medium*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

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

L = label claim (mg/Tablet)

V = volume of *Medium*, 900 mL

Tolerances: See *Table 4*.

Table 4

Level	Number of Tablets Tested	Tolerances	
		Tablets Labeled to Contain 75 or 100 mg of Doxycycline	Tablets Labeled to Contain 150 mg of Doxycycline
A_1	6	No individual value exceeds 50% at 20 min.	No individual value exceeds 30% at 20 min.
A_2	6	Average of 12 units ($A_1 + A_2$) is NMT 50% at 20 min, and no individual unit is greater than 65% dissolved.	Average of 12 units ($A_1 + A_2$) is NMT 30% at 20 min, and no individual unit is greater than 45% dissolved.
A_3	12	Average of 24 units ($A_1 + A_2 + A_3$) is NMT 50% at 20 min, and no individual unit is greater than 65% dissolved.	Average of 24 units ($A_1 + A_2 + A_3$) is NMT 30% at 20 min, and no individual unit is greater than 45% dissolved.

Buffer stage

Conduct this stage of testing on separate Tablets, selecting those that were not previously subjected to the *Acid stage* testing.

Medium: pH 5.5 neutralized phthalate buffer (see *Reagents, Indicators, and Solutions—Solutions, Buffer Solutions*); 1000 mL

Apparatus 1: 50 rpm

Time: 60 min

Standard solution: Prepare the solutions from USP Doxycycline Hyclate RS in *Medium* as directed in *Table 5*. Calculate the concentration, C_S, in mg/mL, of doxycycline using the designated potency, in µg/mg, of doxycycline in USP Doxycycline Hyclate RS.

Table 5

Tablet Strength (mg/Tablet)	Concentration of Doxycycline (mg/mL)
75	0.1
100	0.1
150	0.15

Sample solution: Pass portions of the solution under test through a suitable filter.

Detector: UV 345 nm

Cell: 0.2 cm

Blank: *Medium*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

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

L = label claim (mg/Tablet)

V = volume of *Medium*, 1000 mL

Tolerances: See *Table 6*.

Table 6

Tablets Labeled to Contain 75 or 100 mg of Doxycycline	Tablets Labeled to Contain 150 mg of Doxycycline
NLT 80% (Q) of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) is dissolved.	NLT 70% (Q) of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) is dissolved.

Test 4: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 4*. Proceed as directed for *Dissolution ⟨711⟩, Procedure, Apparatus 1 and Apparatus 2, Delayed-Release Dosage Forms, Method B Procedure*.

Acid stage

Medium: 0.06 N hydrochloric acid; 900 mL, degassed

Apparatus 1: 50 rpm

Time: 20 min

Standard solution: 0.1 mg/mL of doxycycline from USP Doxycycline Hyclate RS in *Medium*. Calculate the concentration, C_S, in mg/mL, of doxycycline using the designated potency, in µg/mg, of doxycycline in USP Doxycycline Hyclate RS.

Sample solution: Pass portions of the solution under test through a suitable filter.

Detector: UV 345 nm

Cell: 0.2-cm quartz

Blank: *Medium*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

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

L = label claim (mg/Tablet)

V = volume of *Medium*, 900 mL

Tolerances

Level 1 (6 Tablets tested)**:** No individual value is more than 30% of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) dissolved in 20 min.

Level 2 (6 Tablets tested)**:** NMT 2 individual values of the 12 tested are greater than 30% of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) dissolved in 20 min.

Buffer stage

Conduct this stage of testing on separate Tablets, selecting those that were not previously subjected to the *Acid stage* testing.

Medium: pH 5.5 neutralized phthalate buffer (see *Reagents, Indicators, and Solutions—Solutions, Buffer Solutions*); 1000 mL, degassed

Apparatus 1: 50 rpm

Time: 30 min

Standard solution: 0.1 mg/mL of doxycycline from USP Doxycycline Hyclate RS in *Medium*

Sample solution: Pass portions of the solution under test through a suitable filter. Calculate the concentration, C_S, in mg/mL, of doxycycline using the designated potency, in µg/mg, of doxycycline in USP Doxycycline Hyclate RS.

Blank: *Medium*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

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

L = label claim (mg/Tablet)

V = volume of *Medium*, 1000 mL

Tolerances: NLT 75% (Q) of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) is dissolved.

Test 5: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 5*. Proceed as directed for *Dissolution ⟨711⟩, Procedure, Apparatus 1 and Apparatus 2, Delayed-Release Dosage Forms, Method B Procedure*.

Acid stage

Medium: 0.06 N hydrochloric acid; 900 mL

Apparatus 1: 100 rpm

Time: 20 min

Standard solution: 0.06 mg/mL of doxycycline from USP Doxycycline Hyclate RS in *Medium*. Calculate the concentration, C_S, in mg/mL, of doxycycline using the designated potency, in µg/mg, of doxycycline in USP Doxycycline Hyclate RS.

Sample solution: Pass portions of the solution under test through a suitable filter.

Detector: UV 345 nm

Cell: 1.0 cm

Blank: *Medium*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

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

L = label claim (mg/Tablet)

V = volume of *Medium*, 900 mL

Tolerances: See *Table 7*.

Table 7

Level	Number of Tablets Tested	Tolerances
A_1	6	No individual value exceeds 50% at 20 min.
A_2	6	Average of 12 units ($A_1 + A_2$) is NMT 50% at 20 min, and no individual unit is greater than 65% dissolved.
A_3	12	Average of 24 units ($A_1 + A_2 + A_3$) is NMT 50% at 20 min, and no individual unit is greater than 65% dissolved.

Buffer stage

Conduct this stage of testing on separate Tablets, selecting those that were not previously subjected to the *Acid stage* testing.

Medium: pH 5.5 neutralized phthalate buffer (see *Reagents, Indicators, and Solutions—Solutions, Buffer Solutions*); 900 mL

Apparatus 1: 100 rpm

Time: 30 min

Standard solution: 0.06 mg/mL of doxycycline from USP Doxycycline Hyclate RS in *Medium*. Calculate the concentration, C_S, in mg/mL, of doxycycline using the designated potency, in µg/mg, of doxycycline in USP Doxycycline Hyclate RS.

Sample solution: Pass portions of the solution under test through a suitable filter.

Blank: *Medium*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

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

L = label claim (mg/Tablet)

V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (Q) of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) is dissolved.

- **Uniformity of Dosage Units ⟨905⟩:** Meet the requirements

IMPURITIES

- **Organic Impurities**

Protect solutions containing doxycycline from light.

Mobile phase, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay*.

System suitability stock solution 1: 1 mg/mL each of USP Doxycycline Related Compound A RS and USP Methacycline Hydrochloride RS in *Diluent*

System suitability stock solution 2: 1.2 mg/mL of USP Doxycycline Hyclate RS in *Diluent*

System suitability solution: Transfer 5 mL of *System suitability stock solution 2* to a 25-mL volumetric flask, heat on a steam bath for 60 min, and evaporate to dryness on a hot plate, taking care not to char the residue. Dissolve the residue in *Diluent*, add 0.5 mL of *System suitability stock solution 1*, and dilute with *Diluent* to volume. Pass the solution through a suitable filter and use the filtrate. This solution contains a mixture of 4-epidoxycycline, doxycycline related compound A, methacycline, and doxycycline. [Note—The solution is stable up to 14 days when stored in a refrigerator.]

Sensitivity solution: 2 µg/mL of USP Doxycycline Hyclate RS in *Diluent*

Standard solution: 4.6 µg/mL of USP Doxycycline Hyclate RS in *Diluent*

Sample solution: Nominally 2.0 mg/mL of doxycycline from NLT 20 Tablets prepared as follows. Transfer a suitable portion of finely powdered Tablets to a suitable volumetric flask. Add 80% of the final volume of *Diluent,* sonicate for about 15 min, shake for about 15 min, and dilute with *Diluent* to volume. Pass through a suitable filter of 0.2-µm pore size and use the filtrate for analysis.

System suitability

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

Suitability requirements

Resolution: NLT 1.5 between methacycline and 4-epidoxycycline; NLT 1.5 between 4-epidoxycycline and doxycycline related compound A; NLT 2.0 between doxycycline related compound A and doxycycline, *System suitability solution*

Relative standard deviation: NMT 5.0% for the doxycycline peak, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

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

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

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

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

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

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

P = potency of doxycycline in USP Doxycycline Hyclate RS (µg/mg)

F = conversion factor, 0.001 mg/µg

Acceptance criteria: See *Table 8*. The reporting threshold is 0.1%.

Table 8

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Oxytetracycline[a,b]	0.39	—
Methacycline[b,c]	0.64	—
4-Epidoxycycline[d]	0.79	1.0
Doxycycline related compound A (6-epidoxycycline)[b,e]	0.88	—
Doxycycline	1.0	—
Any individual unspecified impurity	—	0.2

[a] (4*S*,4a*R*,5*S*,5a*R*,6*S*,12a*S*)-4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,6,10,12,12a-hexahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide.

[b] Process impurities that are controlled in the drug substance are not to be reported. They are listed here for information only.

[c] (4*S*,4a*R*,5*S*,5a*R*,12a*S*)-4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methylene-1,11-dioxo-2-naphthacenecarboxamide.

[d] (4*R*,4a*R*,5*S*,5a*R*,6*R*,12a*S*)-4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide. Main degradation product.

[e] (4*S*,4a*R*,5*S*,5a*R*,6*S*,12a*S*)-4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide.

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight, light-resistant containers. Store at controlled room temperature.

• **Labeling:** When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used.

Change to read:

• **USP Reference Standards** ⟨11⟩

USP Doxycycline Hyclate RS

USP Doxycycline Related Compound A RS

[Note—May be available as a free base or a hydrochloride salt.]

6-Epidoxycycline, or (4*S*,4a*R*,5*S*,5a*R*,6*S*,12a*S*)-4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide.

$C_{22}H_{24}N_2O_8$ ▲444.44▲ (ERR 1-Jul-2022)

(4*S*,4a*R*,5*S*,5a*R*,6*S*,12a*S*)-4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide ▲hydrochloride▲ (ERR 1-Jul-2022) ·

$C_{22}H_{24}N_2O_8 \cdot HCl$ ▲480.90▲ (ERR 1-Jul-2022)

USP Methacycline Hydrochloride RS

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

Topic/Question	Contact	Expert Committee
DOXYCYCLINE HYCLATE DELAYED-RELEASE TABLETS	Documentary Standards Support	SM12020 Small Molecules 1

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