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Amiloride Hydrochloride and Hydrochlorothiazide Tablets

DEFINITION

Amiloride Hydrochloride and Hydrochlorothiazide Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of amiloride hydrochloride ($C_6H_8ClN_7O \cdot HCl$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$).

IDENTIFICATION

• **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

Change to read:

• **B.** ▲The UV spectrum of the amiloride peak of the *Sample solution* exhibits maxima and minima at the same wavelengths as that of the *Standard solution*, as obtained in the *Assay*. The UV spectrum of the hydrochlorothiazide peak of the *Diluted sample solution* exhibits maxima and minima at the same wavelengths as that of the *Diluted standard solution*, as obtained in the *Assay*.▲ (USP 1-Aug-2019)

ASSAY

Change to read:

• **Procedure**

Buffer: Dissolve 136 g of monobasic potassium phosphate in 800 mL of water. Adjust with phosphoric acid to a pH of 3.0. Dilute with water to 1000 mL.

Mobile phase: Methanol, water, and *Buffer* (25:71:4)

Standard stock solution: 1.0 mg/mL of USP Amiloride Hydrochloride RS in methanol

Standard solution: 0.1 mg/mL of USP Amiloride Hydrochloride RS and 1 mg/mL of USP Hydrochlorothiazide RS, prepared as follows. Transfer 10.0 mL of the *Standard stock solution* to a 100-mL volumetric flask containing 100 mg of USP Hydrochlorothiazide RS and 20.0 mL of methanol. Add 4.0 mL of 1 N hydrochloric acid, and dilute with water to volume.

▲**Diluted standard solution:** 0.005 mg/mL of USP Amiloride Hydrochloride RS and 0.05 mg/mL of USP Hydrochlorothiazide RS, from the *Standard solution*, diluted with water▲ (USP 1-Aug-2019)

Sample solution: ▲Nominally 0.1 mg/mL of amiloride hydrochloride and 1 mg/mL of hydrochlorothiazide, prepared as follows.▲ (USP 1-Aug-2019) Transfer an equivalent to 5 mg of amiloride hydrochloride ▲and 50 mg of hydrochlorothiazide▲ (USP 1-Aug-2019) from powdered Tablets (NLT 20) to a 50-mL volumetric flask. Add 15.0 mL of methanol and 2.0 mL of 1 N hydrochloric acid. Sonicate for 10 min, dilute with water to volume, sonicate for an additional 10 min, and filter.

▲**Diluted sample solution:** Nominally 0.005 mg/mL of amiloride hydrochloride and 0.05 mg/mL of hydrochlorothiazide, from the *Sample solution*, diluted with water▲ (USP 1-Aug-2019)

Chromatographic system

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

Mode: LC

Detector: UV 286 nm. ▲For *Identification B*, use a diode array detector in the range of 200–400 nm.▲ (USP 1-Aug-2019)

Column: 3.9-mm × 30-cm; ▲10-µm▲ (USP 1-Aug-2019) packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

▲**Run time:** NLT 2 times the retention time of amiloride▲ (USP 1-Aug-2019)

System suitability

Sample: *Standard solution*

[Note—The relative retention times for hydrochlorothiazide and amiloride ▲▲ (USP 1-Aug-2019) are about 0.7 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between hydrochlorothiazide and amiloride ▲▲ (USP 1-Aug-2019)

Relative standard deviation: NMT 2.0% for hydrochlorothiazide and amiloride

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of amiloride hydrochloride ($C_6H_8ClN_7O \cdot HCl$) in the portion of Tablets taken:

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

r_U = peak response of amiloride ▲▲ (USP 1-Aug-2019) from the *Sample solution*

r_S = peak response of amiloride ▲▲ (USP 1-Aug-2019) from the *Standard solution*

C_S = concentration of USP Amiloride Hydrochloride RS ▲▲ (USP 1-Aug-2019) in the *Standard solution* (mg/mL)

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

Calculate the percentage of the labeled amount of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) in the portion of Tablets taken:

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

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

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

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

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

Acceptance criteria: 90.0%–110.0% of the labeled amount of amiloride hydrochloride ($C_6H_8ClN_7O \cdot HCl$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$)

PERFORMANCE TESTS

Change to read:

• **Dissolution** ⟨711⟩

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Time: 30 min

▲Determine the percentage of the labeled amount of amiloride hydrochloride and hydrochlorothiazide dissolved using the *Spectrophotometric procedure* or the *Chromatographic procedure*.

Spectrophotometric procedure ▲ (USP 1-Aug-2019)

Amiloride standard solution: ▲0.005 mg/mL of USP Amiloride Hydrochloride RS, prepared as follows. Transfer 52 mg of USP Amiloride Hydrochloride RS to ▲ (USP 1-Aug-2019) a 200-mL volumetric flask. Dissolve in and dilute with methanol to volume. Transfer 2.0 mL of this solution to a 100-mL volumetric flask, and dilute with *Medium* to volume.

Hydrochlorothiazide standard solution: 0.01 mg/mL of USP Hydrochlorothiazide RS, prepared as follows. Transfer 100 mg of USP Hydrochlorothiazide RS to a 100-mL volumetric flask. Dissolve in and dilute with methanol to volume. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, and dilute with *Medium* to volume. Transfer 10.0 mL of the resulting solution to a 50-mL volumetric flask, and dilute with *Medium* to volume.

Sample solution A: Pass a portion of the solution under test through a glass fiber filter of 0.45-µm pore size.

Sample solution B: Transfer 5.0 mL of *Sample solution A* to a 25-mL volumetric flask, and dilute with *Medium* to volume.

Blank: *Medium*

Instrumental conditions

Mode: UV-Vis

Analytical wavelengths: 363 nm for amiloride hydrochloride; 270 nm for hydrochlorothiazide

Analysis

Samples: *Amiloride standard solution*, *Hydrochlorothiazide standard solution*, *Sample solution A*, and *Sample solution B*

Calculate the percentage ▲of the labeled amount ▲ (USP 1-Aug-2019) of amiloride hydrochloride ($C_6H_8ClN_7O \cdot HCl$) dissolved:

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

A_U = absorbance of *Sample solution A*

C_S = concentration of ▲USP Amiloride Hydrochloride RS in ▲ (USP 1-Aug-2019) the *Amiloride standard solution* (mg/mL)

V = volume of *Medium*, 900 mL

A_S = absorbance of the *Amiloride standard solution*

L = label claim of amiloride ▲hydrochloride ▲ (USP 1-Aug-2019) (mg/Tablet)

▲Calculate F, the ratio of absorbance of the *Amiloride standard solution* at 270 nm to that at 363 nm:

$$\text{Result} = A_{S270}/A_{S363}$$

A_{S270} = absorbance of the *Amiloride standard solution* at 270 nm

A_{S363} = absorbance of the *Amiloride standard solution* at 363 nm

Calculate A_{UC}, the absorbance of *Sample solution B* at 270 nm, corrected for the interference of amiloride:

$$\text{Result} = A_{U270} - [(A_{U363} \times F)/D]$$

A_{U270} = absorbance of *Sample solution B* at 270 nm

A_{U363} = absorbance of *Sample solution A* at 363 nm

F = ratio of the absorbance of the *Amiloride standard solution* at 270 nm to that at 363 nm

D = dilution factor of *Sample solution B*, 5 ▲(USP 1-Aug-2019)

Calculate the percentage ▲of the labeled amount▲ (USP 1-Aug-2019) of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) dissolved:

$$\text{Result} = [(A_{UC} \times C_S \times V \times D)/(A_S \times L)] \times 100$$

A_{UC} = corrected absorbance of *Sample solution*▲*B*▲ (USP 1-Aug-2019) at 270 nm

C_S = concentration of ▲USP Hydrochlorothiazide RS in▲ (USP 1-Aug-2019) the *Hydrochlorothiazide standard solution* (mg/mL)

V = volume of *Medium*, 900 mL

D = dilution factor of *Sample solution B*, 5

A_S = absorbance of the *Hydrochlorothiazide standard solution*

L = label claim of hydrochlorothiazide (mg/Tablet)

▲Chromatographic procedure▲ (USP 1-Aug-2019)

Buffer and **Mobile phase:** Prepare as directed in the *Assay*.

Standard stock solution: Use the *Standard solution* from the *Assay*.

Standard solution: 0.005 mg/mL of USP Amiloride Hydrochloride RS and 0.05 mg/mL of USP Hydrochlorothiazide RS, from the *Standard stock solution*, diluted with *Medium*

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

Chromatographic system

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

Mode: LC

Detector: UV 286 nm

Column: 4.6-mm × ▲15-cm; 5-µm▲ (USP 1-Aug-2019) packing L1

Flow rate: 1 mL/min

Injection volume: 50 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 2.0 ▲▲ (USP 1-Aug-2019) between hydrochlorothiazide and amiloride

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the ▲percentage of the labeled▲ (USP 1-Aug-2019) amount of amiloride hydrochloride ($C_6H_8ClN_7O \cdot HCl$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) dissolved:

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

r_U = peak response of amiloride or hydrochlorothiazide from the *Sample solution*

r_S = peak response of amiloride or hydrochlorothiazide from the *Standard solution*

C_S = concentration of ▲USP Amiloride Hydrochloride RS or USP Hydrochlorothiazide RS▲ (USP 1-Aug-2019) in the *Standard solution* (mg/mL)

L = label claim of amiloride hydrochloride or hydrochlorothiazide (mg/Tablet)

V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (Q) of the labeled amount of amiloride hydrochloride ($C_6H_8ClN_7O \cdot HCl$) and NLT 75% (Q) of the labeled amount of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) are dissolved.

Change to read:

• **Uniformity of Dosage Units** ⟨905⟩, *Content Uniformity*: Meet the requirements ▲▲ (USP 1-Aug-2019)

IMPURITIES

Change to read:

• ▲**Limit of Benzothiadiazine Related Compound A**▲ (USP 1-Aug-2019)

Buffer, Mobile phase, and **Sample solution:** Prepare as directed in the *Assay*.

▲**System suitability solution:** Use the *Standard solution* from the *Assay*.▲ (USP 1-Aug-2019)

Standard solution: ▲0.01 mg/mL ▲ (USP 1-Aug-2019) of USP Benzothiadiazine Related Compound A RS in *Mobile phase*

Chromatographic system: ▲Proceed as directed in the *Assay*, except for the *Injection volume*.▲ (USP 1-Aug-2019)

Injection volume: 20 µL. ▲For *System suitability*, use 10 µL.▲ (USP 1-Aug-2019)

System suitability

Sample: ▲*System suitability solution*▲ (USP 1-Aug-2019)

[Note—The relative retention times for hydrochlorothiazide and amiloride ▲▲ (USP 1-Aug-2019) are about 0.7 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between hydrochlorothiazide and amiloride ▲▲ (USP 1-Aug-2019)

Relative standard deviation: NMT 2.0% ▲for hydrochlorothiazide and amiloride▲ (USP 1-Aug-2019)

Analysis

Samples: *Sample solution* and *Standard solution*

Calculate the percentage of benzothiadiazine related compound A in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \text{ ▲▲ (USP 1-Aug-2019)} \times 100$$

r_U = peak response of benzothiadiazine related compound A from the *Sample solution*

r_S = peak response of benzothiadiazine related compound A from the *Standard solution*

C_S = concentration of USP Benzothiadiazine Related Compound A RS in the *Standard solution* ▲(mg/mL)▲ (USP 1-Aug-2019)

C_U = nominal concentration of ▲hydrochlorothiazide▲ (USP 1-Aug-2019) in the *Sample solution* (mg/mL)

▲ ▲ (USP 1-Aug-2019)

Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

Change to read:

• **Packaging and Storage:** Preserve in well-closed containers. ▲Store at controlled room temperature. Protect from light.▲ (USP 1-Aug-2019)

• **USP Reference Standards** ⟨11⟩

USP Amiloride Hydrochloride RS

USP Benzothiadiazine Related Compound A RS

4-Amino-6-chloro-1,3-benzenedisulfonamide.

$C_6H_8ClN_3O_4S_2$ 285.73

USP Hydrochlorothiazide RS

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

Topic/Question	Contact	Expert Committee
AMILORIDE HYDROCHLORIDE AND HYDROCHLOROTHIAZIDE TABLETS	Documentary Standards Support	SM22020 Small Molecules 2
REFERENCE STANDARD SUPPORT	RS Technical Services RSTECH@usp.org	SM22020 Small Molecules 2

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