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Method of Test for Azido Compounds in Sartan Drug Substances - Test of 5-AMBBT

1. Scope

This method is applicable to the determination of 5-(4'-((5-azidomethyl)-2-butyl-4-chloro-1*H*-imidazol-1-yl)methyl)-[1,1'-biphenyl]-2-yl)-1*H*-tetrazole (5-AMBBT) in losartan potassium drug substance.

2. Method

After extraction, 5-AMBBT is determined by high performance liquid chromatography (HPLC).

2.1. Equipment

2.1.1. High performance liquid chromatograph

2.1.1.1. Detector: photodiode array detector

2.1.1.2. Column: Poroshell 120 EC-C18, 2.7 μm, 3.0 mm i.d. × 15 cm, or an equivalent product

2.1.2. Ultrasonicator

2.1.3. Centrifuge: centrifugal force ≥ 3000 × g

2.2. Chemicals

Methanol, HPLC grade

Acetonitrile, HPLC grade

Formic acid, HPLC grade

Deionized water, resistivity ≥ 18 MΩ·cm (at 25°C)

5-(4'-((5-azidomethyl)-2-butyl-4-chloro-1*H*-imidazol-1-yl)methyl)-[1,1'-biphenyl]-2-yl)-1*H*-tetrazole (5-AMBBT), reference standard

2.3. Apparatus

2.3.1. Volumetric flask: 10 mL and 20 mL

2.3.2. Centrifuge tube: 15 mL, PP

2.3.3. Membrane filter: 0.22 μm, PVDF

2.4. Mobile phase

2.4.1. Solvent A

Dilute 1 mL of formic acid with deionized water to 1000 mL, and filter with a membrane filter.

2.4.2. Solvent B

Dilute 1 mL of formic acid with acetonitrile to 1000 mL, and filter with a membrane filter.

2.5. Standard solution preparation

Transfer 10 mg of 5-AMBBT reference standard accurately weighed into

a 20-mL volumetric flask, dissolve and dilute to volume with methanol as the standard stock solution. Store at -20°C. and protect from light. Prior to use, mix appropriate volume of the standard stock solution and dilute with methanol to 0.08-15 μg/mL as the standard solution.

2.6. Sample solution preparation

Transfer 0.10 g of sample accurately weighed to a 10-mL volumetric flask, and add 8 mL of methanol. Mix well, sonicate for 10 min, and dilute with methanol to volume. Transfer the mixture to a 15-mL centrifugal tube, and centrifuge at 3000 ×g for 5 min. Filter the supernatant with a membrane filter, and take the filtrate as the sample solution.

2.7. Identification and quantification

Accurately inject 5 μL of sample solution and standard solution into HPLC separately, and operate according to the following conditions. Identify 5-AMBBT based on the retention time and the absorption spectrum. Calculate the amount (μg/g) of 5-AMBBT in the sample by the following formula:

$$\text{The amount of 5-AMBBT in the sample (μg/g)} = \frac{C \times V}{M}$$

Where:

C: the concentration of 5-AMBBT in the sample solution calculated by the standard calibration curve (μg/mL)

V: the final make-up volume of the sample (mL)

M: the weight of the sample (g)

HPLC operating conditions[1]:

Photodiode array detector: 254 nm

Column: Poroshell 120 EC-C18, 2.7 μm, 3.0 mm i.d. × 15 cm, or an equivalent product

Column temperature: 40°C

Mobile phase: A gradient program of solvent A and solvent B is as follows.

Time (min)	A (%)	B (%)
0 → 12	50 → 37	50 → 63
12 → 13	37 → 37	63 → 63
13 → 14	37 → 0	63 → 100
14 → 17	0 → 0	100 → 100
17 → 18	0 → 50	100 → 50

18 → 23	50 → 50	50 → 50

Flow rate: 0.4 mL/min

Injection volume: 5 µL

Note 1: All the parameters can be adjusted depending on the instruments used if the above conditions are not applicable.

Remark

1. Limit of quantification (LOQ) for 5-AMBBT is 8 µg/g.
2. Further validation should be performed when interference compounds appear in samples.

Reference

1. Gričar, M. and Andrenšek, S. 2016. Determination of azide impurity in sartans using reversed-phase HPLC with UV detection. J. Pharm. Biomed. Anal. 125: 27-32.
2. Hertzog, D. L., McCafferty, J. F., Fang, X., Tyrrell, R. J. and Reed, R. A. 2002. Development and validation of a stability-indicating HPLC method for the simultaneous determination of losartan potassium, hydrochlorothiazide, and their degradation products. J. Pharm. Biomed. Anal. 30: 747-760.

Reference chromatogram

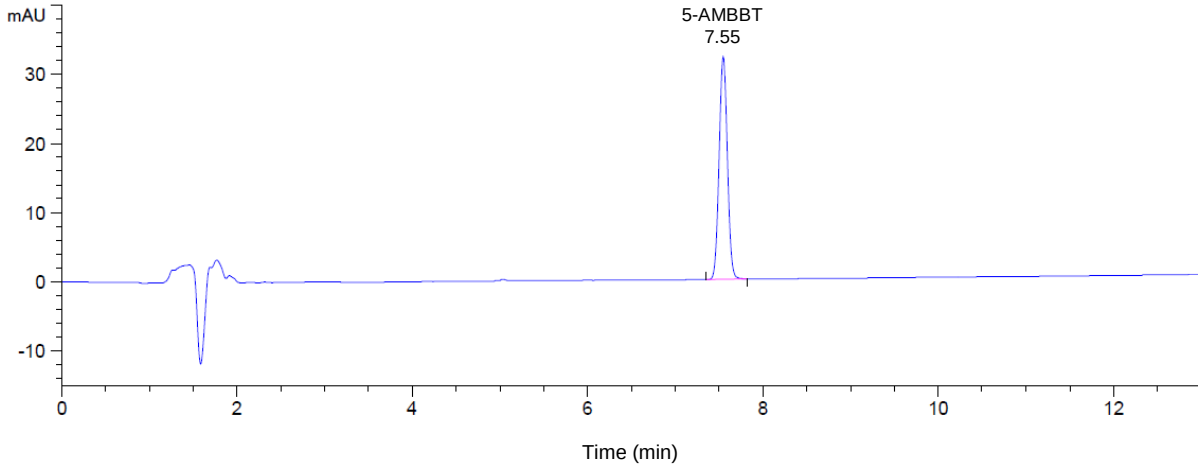

Figure 1. The chromatogram of 5-AMBBT by HPLC