Published, Jul 14, 2021
RA01I006.001

Method of Test for Azido Compounds in Sartan Drug Substances - Test of AZBT

1. Scope

This method is applicable to the determination of 5-(4'-(azidomethyl)-[1,1'-biphenyl]-2-yl)-1*H*-tetrazole (AZBT) in sartan drug substances including candesartan, irbesartan, losartan, olmesartan, and valsartan.

2. Method

After extraction, AZBT is determined by liquid chromatography/tandem mass spectrometry (LC-MS/MS).

2.1. Equipment

2.1.1. Liquid chromatograph/tandem mass spectrometer

2.1.1.1. Ion source: electrospray ionization, ESI.

2.1.1.2. Column: Poroshell 120 Phenyl Hexyl, 2.7 μm, 4.6 mm i.d. × 10 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'-(azidomethyl)-[1,1'-biphenyl]-2-yl)-1*H*-tetrazole (AZBT), reference standard;
5-(4'-(azidomethyl)-[1,1'-biphenyl]-2-yl-2',3',5',6'-d_4)-2*H*-tetrazole, sodium salt (AZBT-d_4), isotope-labeled internal standard.

2.3. Apparatus

2.3.1. Volumetric flask: 10 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. Internal standard solution preparation

Transfer 10 mg of AZBT-d_4 isotope-labelled internal standard accurately weighed into a 10-mL volumetric flask, dissolve and dilute to volume with methanol as the internal standard stock solution. Store in a freezer, and protect from light. When to use, dilute appropriate volume of the internal standard stock solution with methanol to 10 ng/mL as the internal standard solution.

2.6. Standard solution preparation

Transfer 10 mg of AZBT reference standard accurately weighed into a 10-mL volumetric flask, dissolve and dilute to volume with methanol as the standard stock solution. Store in a freezer, and protect from light. When to use, mix appropriate volume of the standard stock solution and the internal standard solution, and dilute with methanol to 1-25 ng/mL (containing 1 ng/mL the internal standard) as the standard solution.

2.7. Standard calibration curve establishment

Accurately inject 10 μL of the standard solution into LC-MS/MS separately, and operate according to the following conditions. Establish the standard calibration curve of AZBT by the ratios of the peak area of AZBT to that of the internal standard vs. the concentrations of AZBT.

LC-MS/MS operating conditions(note):

Column: Poroshell 120 Phenyl Hexyl, 2.7 μm, 4.6 mm i.d. × 10 cm.

Column temperature: 40°C.

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

Time (min)	A (%)	B (%)
0.0 → 1.5	65 → 65	35 → 35
1.5 → 9.0	65 → 35	35 → 65
9.0 → 9.1	35 → 5	65 → 95
9.1 → 12.0	5 → 5	95 → 95
12.0 → 12.1	5 → 65	95 → 35
12.1 → 15.0	65 → 65	35 → 35

Flow rate: 0.5 mL/min.

Inject volume: 10 μL.

Ion spray voltage: -4.5 kV.

Ionization mode: ESI^-.

Ion source temperature: 550°C.

Nebulizer gas, Gas 1: 50 psi.

Heated gas, Gas 2: 60 psi.

Curtain gas: 30 psi.

Collision gas: medium.

Detection mode: multiple reaction monitoring (MRM). Detection ion pair, declustering potential and collision energy are as follows.

Analyte	Ion pair Precursor ion (*m/z*) > Product ion (*m/z*)	Declustering potential (V)	Collision energy (eV)
AZBT	276 > 192*	-65	-16
	276 > 248		-15
AZBT-d_4 (I.S.)	280 > 196	-65	-17

* Quantitative ion pair

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

2. If a divert valve is available, the direction of the mobile phase can be diverted as follows.

Time (min)	Position
0.0 → 6.8	Waste
6.8 → 7.9	Detector
7.9 → 15.0	Waste

2.8. Sample solution preparation

Transfer about 0.025 g of sample accurately weighed to a 10-mL volumetric flask, and add 1 mL of the internal standard solution and 7 mL of methanol. Mix well, sonicate for 30 min, and dilute with methanol to volume. Transfer 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.

2.9. Identification and quantification

Accurately inject 10 μL of sample solution and standard solution into LC-

MS/MS separately, and operate according to the conditions in section 2.7. Identify AZBT based on the retention time and the relative ion intensities(note). Calculate the amount of AZBT in the sample by the following formula:

$$\text{The amount of AZBT in the sample (μg/g)} = \frac{C \times V}{M} \times 10^{-3}$$

Where,

C: the concentration of AZBT in the sample solution calculated by the standard calibration curve (ng/mL)

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

M: the weight of the sample (g)

Note: Relative ion intensities are calculated by peak areas of quantitative ions divided by peak areas of qualitative ions (≤100%). Maximum permitted tolerances of relative ion intensities by LC-MS/MS are as the follows.

Relative ion intensity (%)	Tolerance (%)
> 50	± 20
> 20-50	± 25
> 10-20	± 30
≤ 10	± 50

Remark

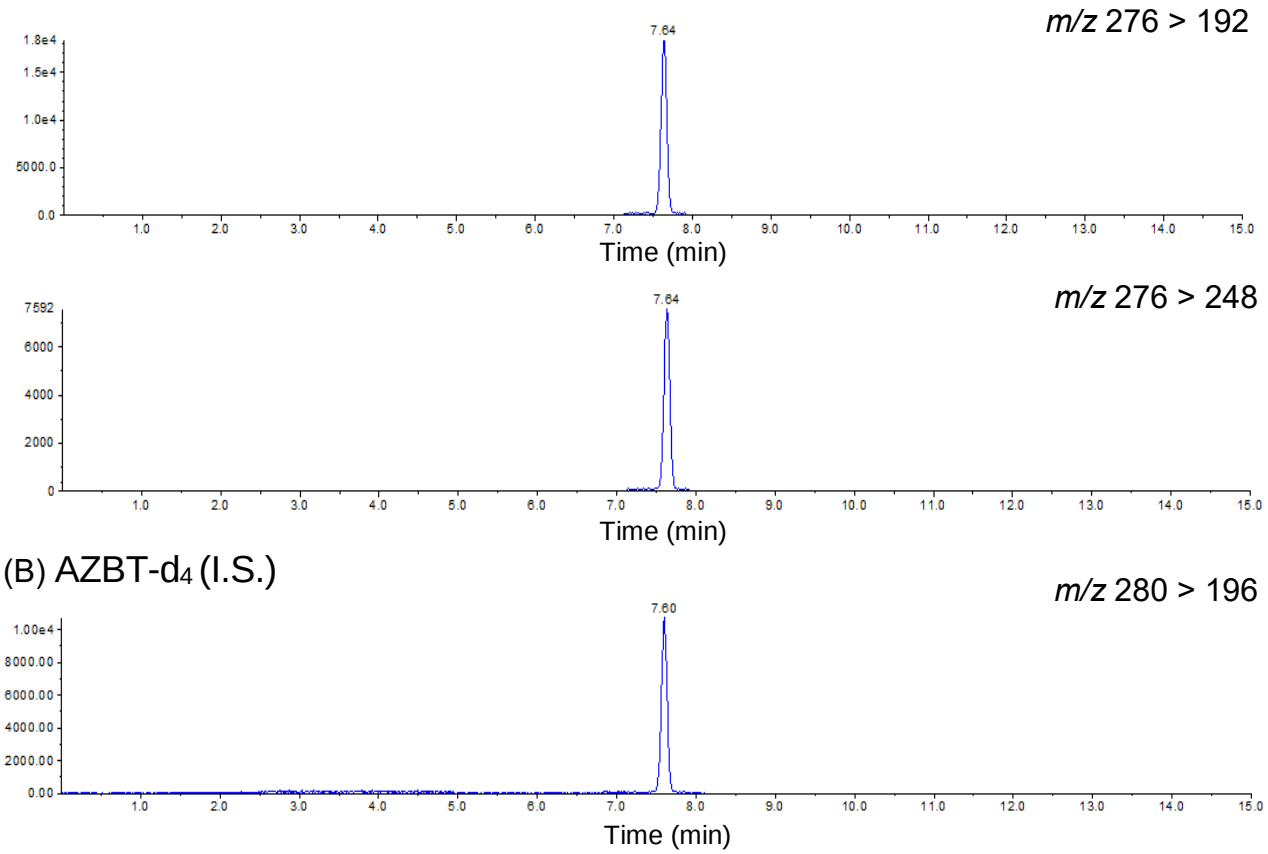
1. Limit of quantification (LOQ) for AZBT is 0.4 μg/g.
2. Further validation should be performed when interference compounds appear in samples.

Reference

1. Dillen, L., Sips, L., de Vries, R., Borgmans, C., Vliegen, M., Leclercq, L. and Cuyckens, F. 2013. Quantitative LC-MS/MS analysis of azide and azidoalanine in in vitro samples following derivatisation with dansyl chloride. Anal. Methods 5: 3136-3141.
2. Ferreirós, N., Dresen, S., Alonso, R. M. and Weinmann, W. 2007. Validated quantitation of angiotensin II receptor antagonists (ARA-II) in human plasma by liquid-chromatography-tandem mass spectrometry using minimum sample clean-up and investigation of ion suppression. Ther. Drug Monit. 29: 824-834.

Reference chromatogram

(A) AZBT



(B) AZBT-d_4 (I.S.)

Figure. The MRM chromatograms of AZBT standard (A) and AZBT-d_4 internal standard (B) analyzed by LC-MS/MS.