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Liquid Chromatograph Mass Spectrophotometer LCMS-8050

Application News

LC-MS/MS Method for Detection and Quantitation of Azido Impurity in Valsartan Drug Substance

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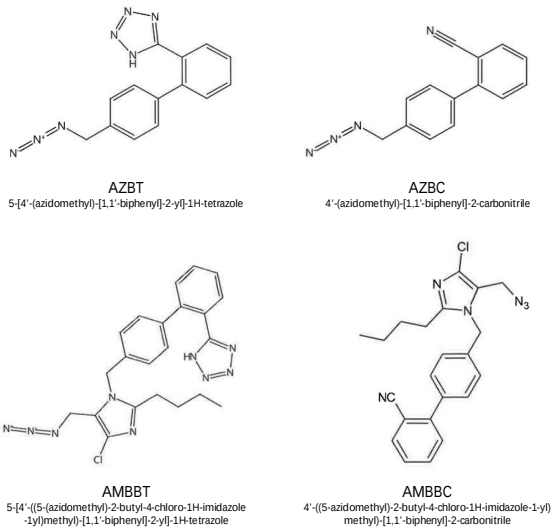
User Benefits

- The method involves the use of LC-MS/MS for the analysis of azido impurity in the sartan drugs.
- The method performances such as linearity, LOD, LOQ, repeatability and recovery were evaluated.
- An MRM based method with superior sensitivity and repeatability helps to ensure reliable laboratory operations.

■ Introduction

Sartan drug substances, including valsartan, losartan, and irbesartan, are primarily used to treat high blood pressure and kidney failure. These block the action of angiotensin II, which regulates blood pressure by narrowing blood vessels and triggering water and salt intake. Recently, sartan drug substances which contained excessive amounts of azido impurities were recalled in some countries.

Azido impurities with azide groups are known to be a mutagenic and potentially carcinogenic substance. Accordingly, the Ministry of Food and Drug Safety in Korea (MFDS) announced the 'AZBT test method for sartan drugs using LC-MS/MS' and provided the guidelines on the method validation results to decide to ensure safety of drugs. This application news describes the evaluation of LC-MS/MS method for analysis of four azido impurities (AZBC, AMBBT, AMBBC and AZBT) in valsartan drug substance (Figure 1).



AZBT
5-[4'-(azidomethyl)-[1,1'-biphenyl]-2-yl]-1H-tetrazole

AZBC
4'-(azidomethyl)-[1,1'-biphenyl]-2-carbonitrile

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

AMBBC
4'-((5-azidomethyl)-2-butyl-4-chloro-1H-imidazole-1-yl) methyl)-[1,1'-biphenyl]-2-carbonitrile

Figure 1 Chemical structure of four azido impurities

■ Analytical method

The sample preparation method and analytical conditions referred to the test method of the Ministry of Food and Drug Safety [1] and the analysis method of OMCL (Official Medicines Control Laboratory), an independent research institute of Swissmedic [2].

Sample preparation

100 mg valsartan was taken into the conical tube and water / acetonitrile (20 / 80) solvent of 100 mL was added. The sample was vortexed until dissolved and then centrifuged at 4,000 rpm for 10 minutes. Take the supernatant and inject 5 µL into the LC-MS/MS.

Analytical condition

Shimadzu Nexera™ X3 LC system and LCMS-8050 mass spectrometer were used as analytical instruments. Table 1 shows the instrumental analytical conditions and MRM conditions for the analysis of four azido impurities.

Table 1. Instrumental analysis condition

Liquid chromatograph Nexera X3	
Flow rate	0.4 mL/min
Mobile Phase	A) 0.1% Formic acid in water B) 0.1% Formic acid in 95% acetonitrile
Gradient	B 35% (0 min) – B 40% (5.5 min) – B 100% (12-14 min) – B 35% (14.01-18 min)
Diverter valve	0 -7.6 min (to waste), 7.6 - 9.0 min (to MS), 9.0 - 9.6 min (to waste), 9.6 - 18 min (to MS)
Column	Shim-pack™ GIST C18 (3.0 x 100 mm., 3 µm) (P/N: 227-30009-05)
Column Temp.	40 ˚C
Injection Volume	5 µL
Detector	SPD-40 (254 nm)
Mass spectrometer LCMS-8050	
Ionization method	ESI (Positive)
Nebulizing Gas Flow	3 L/min
Heating Gas Flow	10 L/min
Drying Gas Flow	10 L/min
Interface Temp.	300 ˚C
DL Temp.	250 ˚C
Heat Block Temp.	400 ˚C

MRM conditions

Name	Precursor Ion (m/z)	Product Ion 1 (m/z)	Q1 (V)	Collision Energy (V)	Q3 (V)
AZBT	278	235	-10	-9	-25
AZBC	207	179	-13	-23	-17
AMBBT	448	405	-10	-11	-19
AMBBC	405	192	-14	-22	-18

■ Results and Discussion

Separation of the four azido impurities and valsartan

Through the LC analysis conditions in Table 1, the valsartan and four azido impurities were separated, and the results are shown in Figure 2. Divert valve program was developed in order to send only the four azido impurities into the mass spectrometer for highly sensitive detection while delivering the high amount of drug substance to waste in order to avoid mass spectrometer contamination.

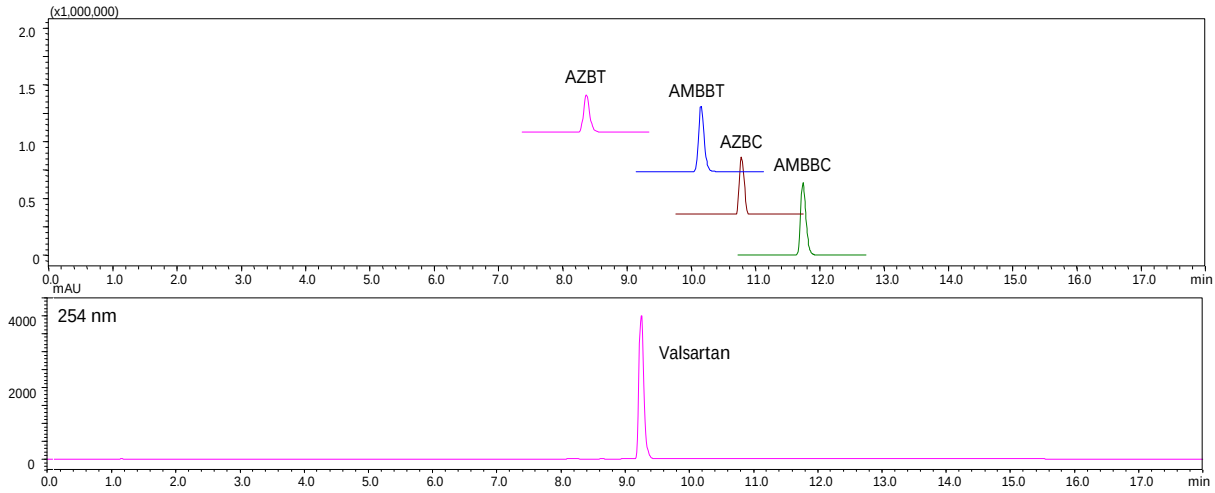


Figure 2. MS chromatogram of four azido impurities(Up) and UV chromatogram of valsartan(Down)

Linearity

Four azido impurity standards were dissolved in 80% acetonitrile to prepare the standard stock solution of 1 µg/mL. The calibration curve of standard solution was prepared from the standard stock solution at each final concentration (0.5-50 ng/mL) using 80% acetonitrile as a diluent. As shown in Figure 3, for all compounds, R^2 showed excellent linearity greater than 0.99. The limit of detection (LOD) and the limit of quantification (LOQ) were calculated as S/N=3 and S/N=10 using LabSolutions™. The LOQ was between 0.03 to 0.5 ng/mL level depending on each compound and is shown in Table 2, respectively.

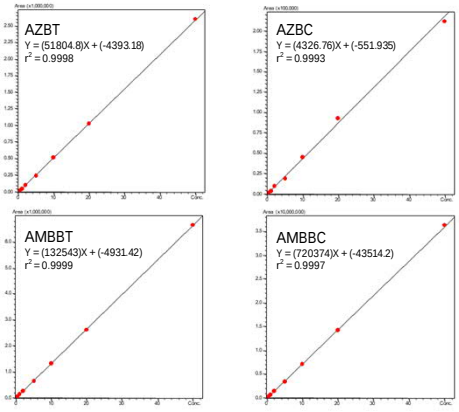


Figure 3 Calibration for four azido impurities

Table 2 LOD and LOQ of four azido impurities

Conc. (ng/mL)	AZBT	AZBC	AMBBT	AMBBC
LOD	0.03	0.2	0.03	0.01
LOQ	0.1	0.5	0.1	0.03

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Recovery

The recovery for the four azido impurities was evaluated using blank valsartan with azido impurity standards mixtures at low level (1 µg/kg), medium level (20 µg/kg) and high level (40 µg/kg). The recovery ratio was calculated as the average concentration obtained by analyzing the three replicates prepared for each concentration. The recovery results were around 93.0 to 105.3% as shown in Table 3.

Table 3 Recovery results for four azido impurities(%), *n=3*

Concentration	AZBT	AZBC	AMBBT	AMBBC
Low (1 µg/g)	105	98	103	98
Mid (20 µg/g)	100	105	98	93
High (40 µg/g)	101	101	99	94
Average of recovery (%)	102	101	100	95

■ Conclusion

Using the Shimadzu LCMS-8050 system, an LC-MS/MS method was developed and evaluated for the quantification of four azido impurities (AZBT, AZBC, AMBBT, AMBBC) in Valsartan. Linearity, LOD, LOQ, and recovery ratio were selected for four impurities as evaluation items, and linearity had an excellent correlation coefficient of 0.99 or more for all compounds. LOD and LOQ were at the levels of 0.01 to 0.2 ng/mL and 0.03 to 0.5 ng/mL, respectively, and the recovery ratio of each compound at low, medium, and high concentrations was excellent at 93 to 105%.

■ References

1) Food and Drug Safety in Korea, AZBT test method for sartan drugs using LC-MS/MS (2021.08)
2) Genotoxic substances in sartans, OMCL Swissmedic (2021)

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