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Liquid Chromatograph Mass Spectrophotometer LCMS-8050

Application News

A Sensitive LC-MS/MS Method for the Analysis of Azido Impurity in Losartan Drugs

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User Benefits

- The highly sensitive LC-MS/MS method for the detection and quantification of azido impurities in the losartan drug substances was developed.
- Four azido impurities were evaluated for linearity, LOD, LOQ, repeatability and recovery on the LCMS-8050 triple quadrupole mass spectrometer coupled with a Nexera™ X3 LC system.

■ Introduction

Sartan drugs such as valsartan, losartan, and Irbesartan are high blood pressure treatments that narrow blood vessels and block the action of angiotensin II, which regulates blood pressure by causing water and salt intake. The recent discovery of N-nitrosamine impurities known as potent carcinogens in several sartan drug substances has become a global issue. In addition, the AZBT that is an azido impurity known as a mutagenic and potentially carcinogenic substance, was also detected in sartans. As a result, the European Medicine Agency(EMA) and Health Canada(HC), which are responsible for supervising medicines, have recalled some sartan drugs. Besides to AZBT, there are various types of azido impurities such as AZBC, AMBBT, and AMBBC as shown in Fig. 1.

In this regard, the Ministry of Food and Drug Safety in Korea (MFDS) has announced the 'Test method for AZBT in sartan drug substances using LC-MS/MS' for the analysis of AZBT, one of the azido impurities. Also, in Europe, European Directorate for the Quality of Medicines & HealthCare (EDQM) suggested the test method, 'AZBT impurity in Valsartan, Irbesartan, Losartan, Candesartan'.

In addition, the official Medicines Control Laboratory(OMCL), the research institute from the Swissmedic, introduced 'AZBT and AZBC test methods for sartan-type drug substances using LC-MS/MS' based on EDQM's test method.

Accordingly, this application news describes the evaluation of LC-MS/MS method for analysis of four azido impurities (AZBC, AMBBT, AMBBC and AZBT) in losartan drug substance.

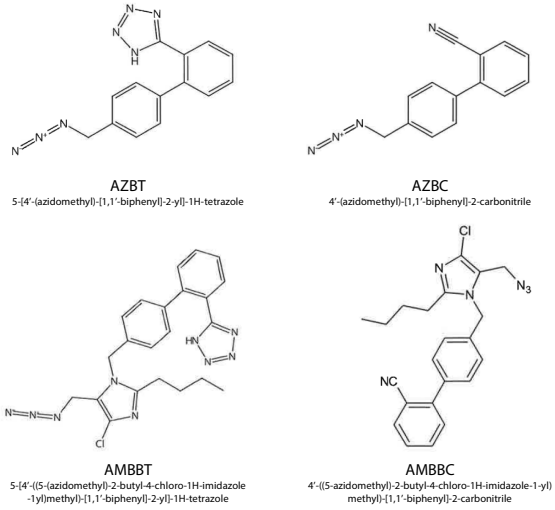

Fig. 1. Chemical structure of four azido impurities

■ Analytical method

The sample preparation method and analytical conditions referred to the test method of the MFDS[1], EDQM[2] and OMCL[3].

Sample preparation

100 mg of losartan was taken into the conical tube, then water : acetonitrile (20 : 80 v/v) diluent of 100 mL was added. The sample was vortexed till it dissolved and then centrifuge at 4,000 rpm for 10 minutes. Take the supernatant and inject the 5 µL to LC-MS/MS.

Analytical condition

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

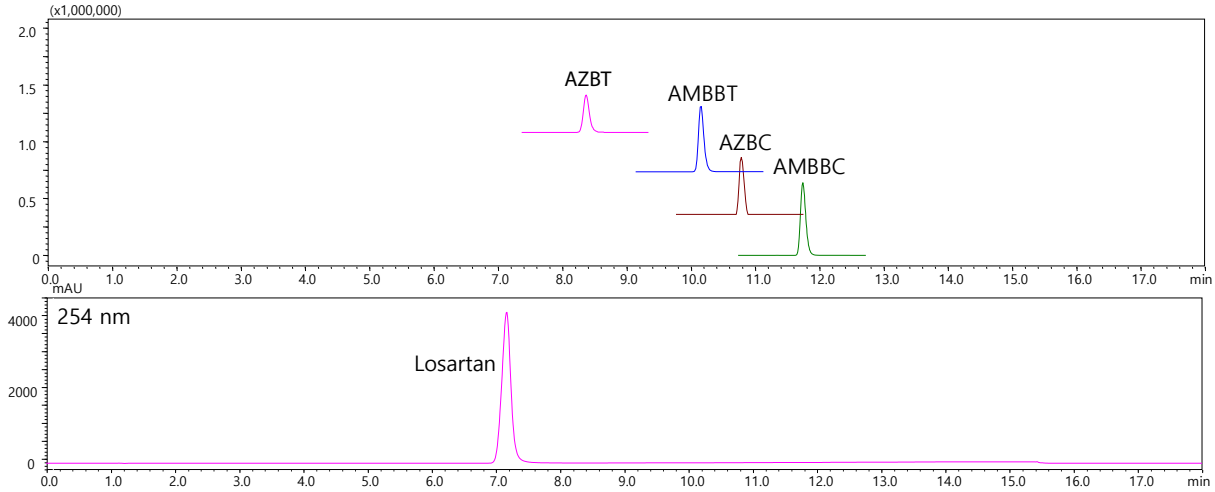

Fig. 2 MS chromatogram of four azido impurities(Up) and UV chromatogram of losartan(Down)

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 - 18.0 min)
Diverter valve	0 - 7.6 min (to waste), 7.6 - 13.0 min (to MS),
Column	Shim-pack™ GIST C18 (3.0 I.D. 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 losartan

Through the LC analysis conditions in Table 1, the losartan and four azido impurities were separated, and the results are shown in Fig. 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 substances to waste in order to avoid mass spectrometer contamination.

Linearity

Four azido impurity standards were dissolved in 80 % acetonitrile to make the standard stock solution with a concentration of 1 µg/mL. A series of calibration standards were prepared from above the solution using 80 % acetonitrile as diluent to obtain the final concentration (0.5 - 50 ng/mL). Excellent linearity with r^2 greater than 0.99 for all compounds was achieved (Fig. 3). The limit of detection(LOD) and the limit of quantification(LOQ) were calculated using LabSolutions™ as S/N=3 and S/N=10. LOQ was between 0.03 to 0.5 ng/mL level according to the respective compound (Table 2).

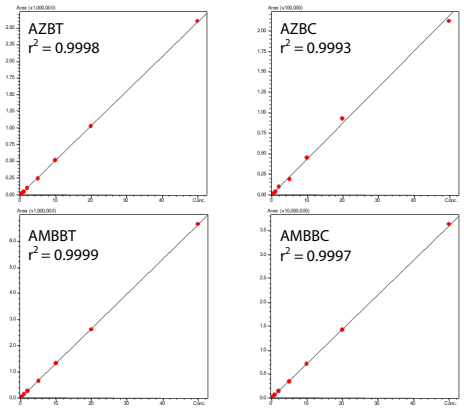

Fig. 3 Calibration curve for four azido impurities

Table 2. LOD and LOQ for 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

Recovery

The recovery rate for four azido impurities was evaluated using blank losartan spiked with mixture of azido impurity standards at levels of 1 µg/kg (low), 20 µg/kg(medium) and 40 µg/kg (high). The recovery ratio was calculated as the average concentration obtained by analyzing three replicates prepared for each concentration. The recovery results were around (84 - 116) % as shown in Table 3.

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

Concentration	AZBT	AZBC	AMBBT	AMBBC
Low (1 µg/g)	100	105	89	89
Mid (20 µg/g)	96	111	86	86
High (40 µg/g)	94	116	84	84
Average of recovery (%)	97	111	86	86

■ Conclusion

LC-MS/MS method for the quantification of four azido impurities (AZBT, AZBC, AMBBT, AMBBC) in losartan has been evaluated on the Shimadzu LCMS-8050 system. Linearity was performed for the four impurities and all of the correlation coefficients were greater than 0.99. The LOD and LOQ were at the level of (0.01 - 0.2) ng/mL and (0.03 - 0.5) ng/mL, respectively. The recovery ratio for each component at low, medium, and high concentrations was excellent at (84 - 116) %.

■ References

1) Food and Drug Safety in Korea, Test method for AZBT in sartan drugs using LC-MS/MS (2021)
2) European Directorate for the Quality of Medicines and HealthCare, AZBT impurity in Valsartan, Irbesartan, Losartan, Candesartan LC-MS/MS Method (2021)
3) Genotoxic substances in sartans, OMCL Swissmedic (2021)

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