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Application News

Liquid Chromatograph Mass Spectrometer LCMS-8050

LC-MS/MS Method for Detection and Quantitation of Azido Impurities in Irbesartan Drug Substance

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User Benefits

- A method based on the high sensitivity MRM was established for the quantitative determination of azido impurities in irbesartan API with LCMS-8050.
- The performance for established method was evaluated in terms of linearity, LOD, LOQ, recovery and the reliable results were obtained.

■ Introduction

The azido impurity, azidomethyl biphenyl tetrazole (AZBT), known as a mutagenic impurity was detected in sartan-type antihypertensive drug. As a result, drug recalls were carried out by regulatory agencies such as European Medicine Agency (EMA), Medicines and Healthcare products Regulatory Agency (MHRA) and Health Canada. Accordingly, the Ministry of Food and Drug Safety (MFDS) in Korea distributed the LC-MS/MS method for the determination of AZBT in sartan drug substances as a guideline[1] and strives to secure drug safety.

Azido impurities can be formed during the synthesis of sartan active pharmaceutical ingredient (API) containing tetrazole rings. In sartan, the tetrazole ring is formed by the reaction between appropriate nitrile and azido groups, which can be accompanied by trace levels of azide impurities as a by-product. There are various types of azido impurities besides AZBT, which is a domestically regulated component. Accordingly, this Application News is intended to present the evaluation of LC-MS/MS method for analysis of 4 azido impurities in irbesartan drug substance (Figure 1).

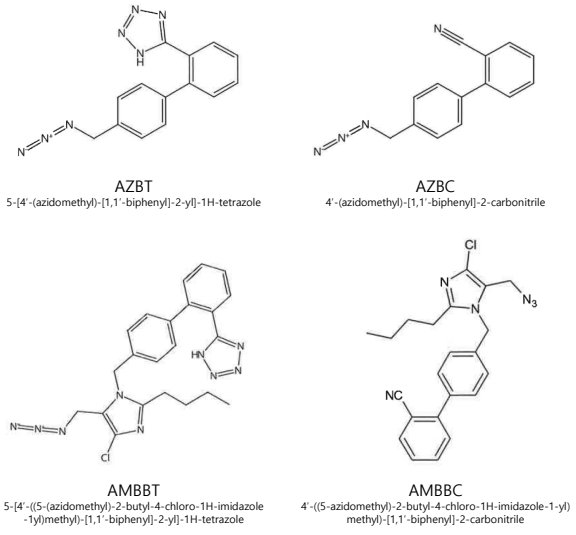

Figure 1 Chemical Structure of Azido Impurities as a Target Compound

■ Measurement Conditions

The analytical method was performed in reference to the OMCL (Official Medicines Control Laboratory) method[2]. The instrumental conditions and MRM conditions for each compound are shown in Table 1 and Table 2.

Table 1 Instrumental Conditions

Liquid chromatograph	Nexera™ X3
Column	: Shim-pack™ GIST C18 (3.0 mm I.D. x 100 mm L., 3 µm)
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) – 100 % (12-14 min) – B 35 % (14.01-18 min)
Diverter valve	: 0 – 7.6 min (waste), 7.6 – 18 min (MS)
Oven temp.	: 40°C
Injection volume	: 5 µL
Detector	: UV 254 nm
Mass spectrometer	LCMS-8050
Interface	: ESI
MS Mode	: Positive mode
Interface temp.	: 300°C
DL Temp.	: 250°C
Heat block Temp.	: 400°C
Nebulizing Gas Flow	: Nitrogen, 3.0 L/min
Drying Gas Flow	: Nitrogen, 10.0 L/min
Heating Gas Flow	: Zero Air, 10.0 L/min

Table 2 Multiple Reaction Monitoring (MRM) Conditions

Name	Precursor *m/z*	Product *m/z* (1)	Product *m/z* (2)
AZBT	278	235	207
AZBC	207	179	151
AMBBT	448	405	207
AMBBC	405	192	165

■ Sample Pretreatment

The sample pretreatment procedure was performed in reference to the MFDS method[1]. Irbesartan API was simply pretreated through the process of dissolution and centrifugation as shown in Figure 2.

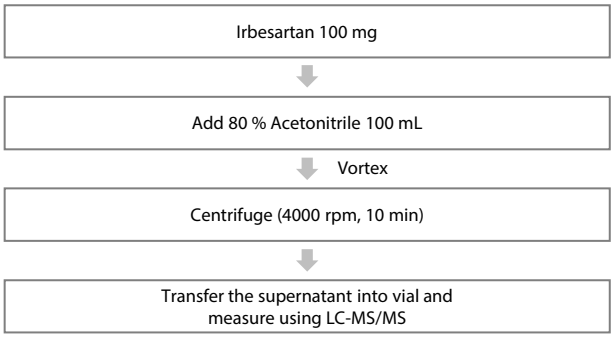

Figure 2 Sample Preparation Protocol

■ Results and Discussion

Separation of Irbesartan API and Azido Impurities

The separation of Irbesartan API and four azido impurities was optimized as shown in Figure 3.

To eliminate interference from matrix and possible contamination of mass spectrometer due to high concentration of API, a suitable liquid chromatography method was applied along with the divert valve function.

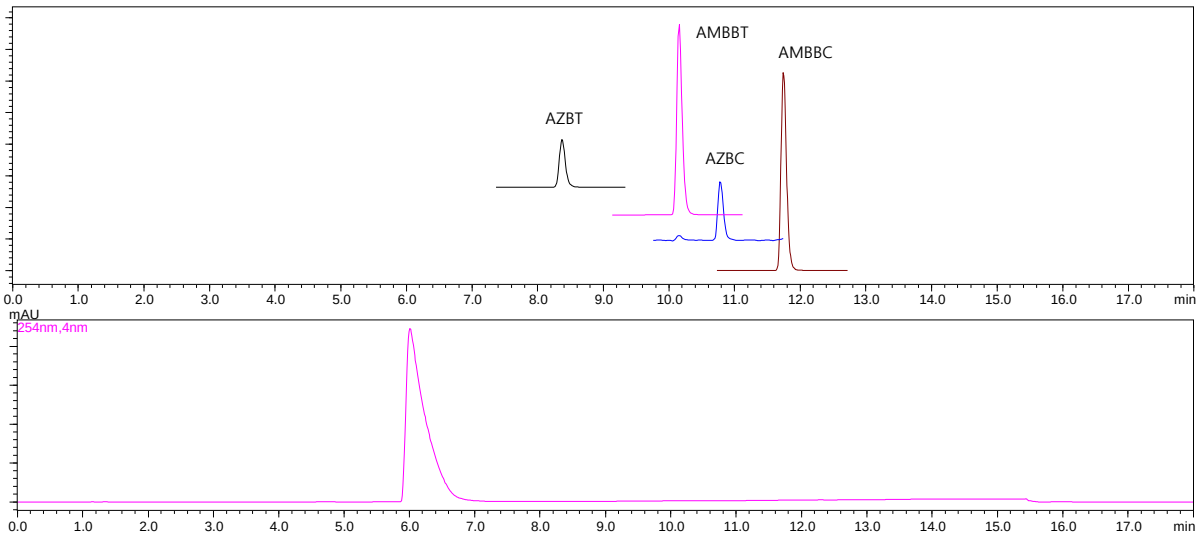

Figure 3 MS Chromatogram of Four Azido Impurities (top) and UV Chromatogram of Irbesartan API (bottom)

Calibration curve and LOD and LOQ

The standard solution with four azido impurities for the calibration curve were prepared by dissolving in 80% acetonitrile. Good linearity was obtained in the range of calibration curve from 0.5 ng/mL to 50 ng/mL and the correlation coefficients (r^2) for each compound was 0.99 or more as shown in Figure 4.

The limit of detection (LOD) and limit of quantitation (LOQ) were calculated using LabSolutions™ software as S/N=3 and S/N=10. The obtained detection limit ranged from 0.03 ng/mL to 0.2 ng/mL depending on the compounds (Table 3).

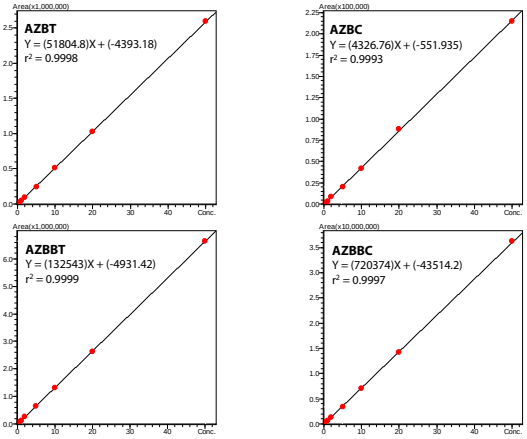

Figure 4 Calibration Curve of Four Azido Impurities

Table 3 LOD and LOQ of Azido Impurities

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

Spike-and-Recovery Test

The spike-and-recovery test was conducted using the irebesartan API sample. The spiked concentrations in each sample were in three levels; 1 µg/g (low level) and 20 µg/g (medium level) and 40 µg/g (high level). Table 4 shows that the obtained spike-and-recovery test results were from 95 % to 111 % in these measurements. The results were calculated as the average concentration of 3 samples for each concentration.

Table 4 Recovery (%) of Irbesartan API spiked with Azido Impurities, *n*=3

Recovery test sample	AZBT	AZBC	AMBBT	AMBBC
Low level (1 µg/g)	103	111	102	101
Medium level (20 µg/g)	105	104	100	97
High level (40 µg/g)	108	105	101	95
Average	105	107	101	98

■ Conclusion

In this Application News, simultaneous analysis of four azido impurities (AZBT, AZBC, AMBBT, AMBBC) in irbesartan API was carried out using a Nexera X3 and LCMS-8050 system. And we demonstrated excellent performances of our system for linearity and recovery test. The calibration curves for the four azido impurities were excellent with $r^2 > 0.99$ or higher in the range of 0.5 to 50 ng/mL. The limit of detection was at the level from 0.03 ng/mL to 0.2 ng/mL depending on the compounds. The recovery test results were excellent with the range from 95 % to 111 % at three concentration levels.

<Reference>

[1] MFDS Method (2021), https://www.mfds.go.kr/brd/m_218/view.do?seq=33406

[2] Genotoxic substances in sartans, OMCL Swissmedic (2021)

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