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Application News

Liquid Chromatograph Mass Spectrophotometer LCMS-8050

# Quantitative analysis of azido impurity in the sartan drugs using LC-MS/MS

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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 performance such as linearity, LOQ, repeatability and recovery was evaluated in reference to the KFDA method published in 2021.
- An MRM based method with superior sensitivity and repeatability helps to ensure reliable laboratory operations.

Shimadzu LCMS-8050

## ■ Introduction

AZBT (5-(4`-(azidomethyl)-[1,1`-biphenyl]-2yl)-1H tetrazole) is an azido impurity of sartan drugs known to cause gene mutation which increases risks of developing cancer. Irbesartan, valsartan and losartan, which contained excessive amounts of azido impurities have been recalled in Canada in May 2021. Accordingly, the Ministry of Food and Drug Safety in Korea(KFDA) also announced the 'AZBT test method for sartan drugs using LC-MS/MS' and provided the guidelines on the method validation results.[1]

The acceptance criteria for system suitability from KFDA method at 1.0 ng/mL of AZBT standard solution were signal to noise(S/N) ratio≥10, relative standard deviation(RSD) for peak areas(*n*=6)≤10%, coefficients of determination($R^2$)≥0.995 and symmetry factor of peak≤2.0. In this application news, the limit of quantitation(LOQ), linearity, repeatability and recovery for AZBT spiked sartan drugs were evaluated using Shimadzu LCMS-8050 with the KFDA method.

## ■ Analytical conditions

Analytical conditions and Multiple Reaction Monitoring(MRM) transitions of AZBT standard were described in Table 1.

Table 1 Analysis conditions for LC-MS/MS

| **Liquid Chromatography** | |
|---|---|
| System | : Nexera™ X3 |
| Analytical Column | : Shim-pack™ Velox PFPP (2.1 x 150 mm., 2.7 µm) (P/N: 227-32021-04) |
| Flow rate | : 0.4 mL/min |
| Mobile phase | : (A) 0.1 % Formic Acid in Water |
| | : (B) 0.1 % Formic Acid in Methanol |
| Gradient | : 30% B (0 - 2.0 min) - 50% B (2.5 - 11.0 min) - 95% B (11.5 - 12.5) - 30% B(12.6 - 15.0 min) (Waste to 7.2 - 15.0 min) |
| Column Temp. | : 35°C |
| Autosampler Temp. | : 4°C |
| Injection volume | : 5 µL |

| **Mass spectrometry** | |
|---|---|
| System | : LCMS-8050 |
| Ionization method | : ESI (+) |
| Nebulizer gas flow | : 3 L/min |
| Dry gas flow | : 10 L/min |
| Heating gas flow | : 10 L/min |
| Interface temp. | : 300°C |
| Desolvation Line temp. | : 250°C |
| Heat block temp. | : 400°C |

**MRM Condition**

| Target compound | Polarity | Q1 m/z | Q3 m/z | Q1 (V) | Collision Energy (V) | Q3 (V) |
|---|---|---|---|---|---|---|
| AZBT | Positive | 277.9 | 235.1 | -14 | -9 | -25 |
| | | 277.9 | 207.1 | -14 | -15 | -14 |

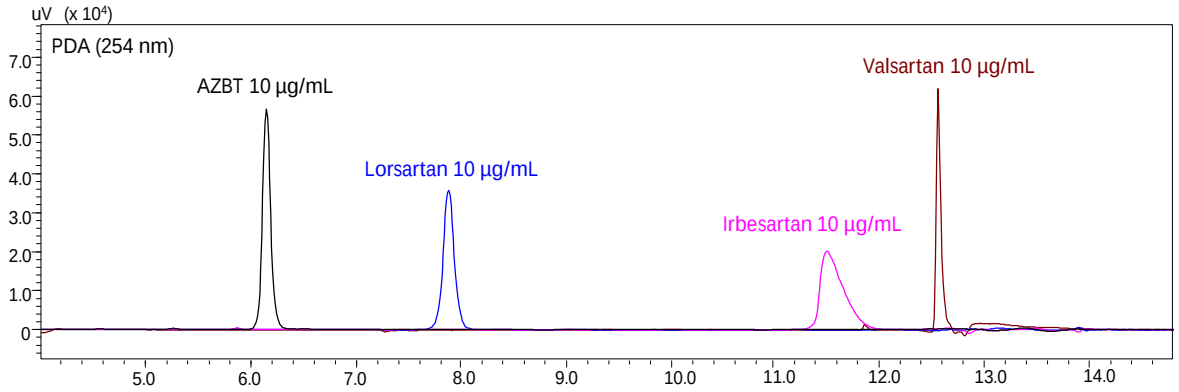


Figure 1 PDA overlay chromatograms of AZBT and sartan drugs

## ■ PDA and MS Chromatograms

In order to checking the chromatographic separation of target peaks, AZBT and sartan drugs were diluted with each 75% methanol and analyzed using a PDA detector under the LC analytical conditions of Table 1. The elution order of AZBT, Irbesartan, Valsartan and Lorsartan was shown in Figure 1. In accordance with elution order, divert valve was employed to direct sartan drugs to waste (7.2 - 15.0 min) and to protect mass spectrometer from the contaminations. Figure 2 shows the MS chromatogram of AZBT standard analyzed under the analytical conditions in Table 1.

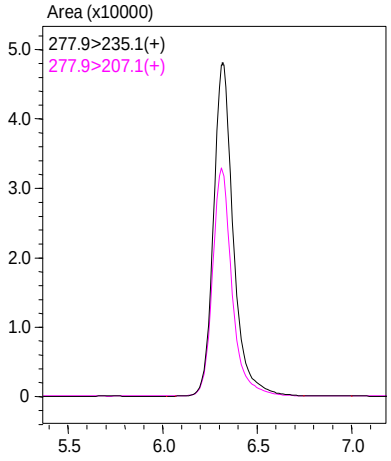


Figure 2 MS chromatogram of AZBT standards (1 ng/mL)

## ■ Linearity and LOQ

1 µg/mL of AZBT standard was prepared in 75 % methanol and then diluted with 75 % methanol to obtain 0.5, 1.0, 2.0, 5.0, 10.0, 20.0, 50.0 ng/mL standard solutions for the calibration curve. After centrifuging the standard solutions for each concentration at 4,000 rpm for 10 minutes, the supernatant was filtered through a 0.2 µm membrane filter (PVDF) and analyzed the linearity of calibration curve.

As a result of the analysis, $R^2$ of the seven-point AZBT calibration curve was 0.999 indicating excellent linearity as shown in Figure 3. LOQ of the AZBT standard calculated by the ratio of 10 for S/N was 0.01 ng/mL and the AZBT MS chromatogram at the LOQ is shown in Figure 4.

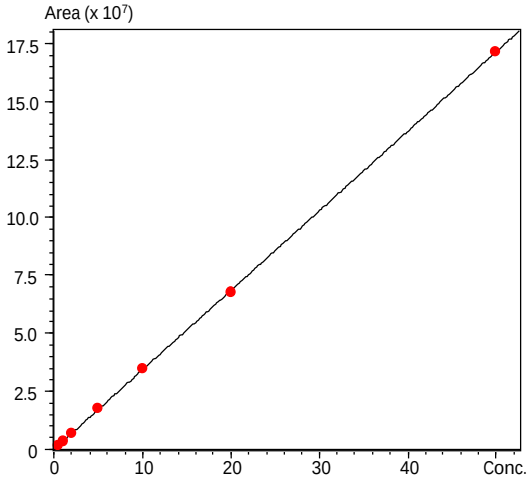


Figure 3 Calibration curve of AZBT (0.5 - 50.0 ng/mL)

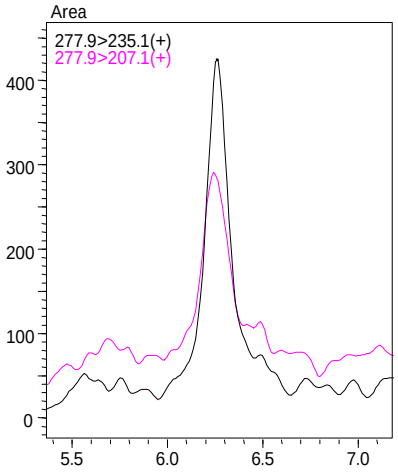


Figure 4 MS chromatogram of AZBT at the LOQ (0.01 ng/mL)

## ■ Repeatability

For evaluating repeatability of LC-MS/MS system, the six individual replicates of 1 ng/mL of AZBT standards were measured. The observed %RSD of peak areas of AZBT standards for repeatability was less than 10%. The coefficients of the peak symmetry was less than 2.0 in all of six analysis. Repeatability results and MS chromatograms of AZBT standards were shown in Table 2 and Figure 5, respectively.

Table 2. Repeatability results for 6 replicates of AZBT (1 ng/mL)

| No. | Area |
|---|---|
| 1 | 342,252 |
| 2 | 341,433 |
| 3 | 339,156 |
| 4 | 339,255 |
| 5 | 332,387 |
| 6 | 336,475 |
| Average | 338,493 |
| STDEV | 3609.7 |
| %RSD | 1.1 |
| Avg. of symmetry factor | 1.3 |

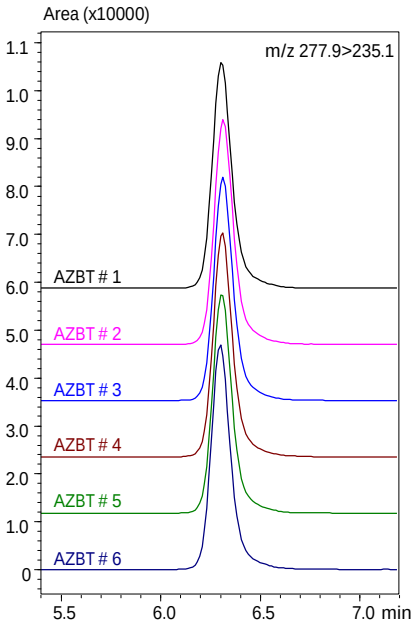


Figure 5. MS chromatograms of AZBT standards (1 ng/mL)

## ■ Recovery

Recovery was evaluated by analyzing six replicates of fortified samples at 1 ng/mL, 5 ng/mL, and 20 ng/mL against calibration curve. For evaluating recovery, 100 mg of sartan drugs irbesartan, valsartan, and losartan were dissolved in 75 % methanol to prepare solution of 1,000 µg/mL. To prepare 1 ng/mL, 5 ng/mL, and 20 ng/mL of AZBT fortified samples, AZBT standard was added to each drug solution. And each of the AZBT spiked samples was centrifuged at 4,000 rpm for 10 minutes and filtered the supernatant with a 0.2 µm membrane filter (PVDF). Mean recoveries were found within 85-105% and the results were shown in Table 3.

Table 3. Recovery results of sartan drugs

| | Recovery(%) (n=6) | | |
|---|---|---|---|
| | 1 ng/mL | 5 ng/mL | 20 ng/mL |
| Irbesartan | 89.6 ± 1.0 | 91.8 ± 0.6 | 91.9 ± 0.2 |
| Valsartan | 102.6 ± 0.6 | 98.9 ± 0.5 | 98.7 ± 0.0 |
| Lorsartan | 89.0 ± 0.9 | 89.4 ± 0.4 | 86.5 ± 0.4 |

## ■ Summary

Based on the KFDA's 'AZBT test method for sartan drugs using LC-MS/MS', the method was evaluated using Shimadzu LCMS-8050 such as linearity, LOQ, repeatability, recovery. As a result, the acceptance criteria of system suitability from the KFDA method the S/N ratio at 1.0 ng/mL (≥10), $R^2$ of the calibration curve (≥ 0.995), the symmetry factor of the peak (≤ 2.0) and the RSD (≤ 10.0%) of peak areas were met.

The calibration curve showed that the $R^2$ was 0.999 in the range of 0.5 – 50 ng/mL, and the LOQ was 0.01 ng/mL for AZBT standard. The repeatability of AZBT for 6 replicates (1 ng/mL) was 1.1 % and the recovery for the sartan drugs was (86.5 – 102.6) %.

Based on the method evaluation results, high-sensitivity quantitative analysis of AZBT in sartan drugs was possible in Shimadzu LCMS-8050 system.

## ■ Reference

1) Food and Drug Safety in Korea, AZBT test method for sartan drugs using LC-MS/MS (2021.08)



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