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Application News

Liquid Chromatography Mass Spectrometry

Robust and Sensitive Azido Impurities Quantitative Analysis in Five Sartan Drug Substances

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■ Summary

This application news demonstrates a highly sensitive and robust method for determining azido impurities (AZBT and AMBBT) in five different sartan drug substances – olmesartan, losartan, irbesartan, valsartan, and candesartan – using a Shimadzu LCMS-8060.

■ Background

Sartans, also known as angiotensin-II-receptor antagonists, belong to a class of medicines used for patients with hypertension (high blood pressure) and those with certain heart or kidney diseases. They work by blocking the action of angiotensin II, a hormone that constricts blood vessels and causes blood pressure to rise. Over the past few years, sartan medications have been recalled due to concerns about N-nitrosodimethylamine (NDMA), a probable carcinogen. However, NDMA is not the only impurity of concern in these medications. As drug companies have addressed the NDMA issue, they have also found new potentially dangerous chemicals known as azido impurities.

Azido impurities are mutagenic, indicating they can alter people's DNA and potentially raise their cancer risk. In early 2021, authorities in Canada and European asked drugmakers to recall certain sartan medications after detecting azido impurities in them.[1,2]

One of the azido impurities, (5-(4' (azidomethyl)-[1,1'-biphenyl]-2-yl)-1H-tetrazole, also known as azidomethyl-biphenyl-tetrazole (AZBT), can form during the manufacturing of the active ingredient in some sartan medications, e.g. olmesartan, losartan, irbesartan, valsartan, and candesartan. Losartan azido impurity, 5-[4'-[(5-(azidomethyl)-2-butyl-4-chloro-1H-imidazol-1-yl)methyl]-[1,1'-biphenyl]2-yl]-1H-tetrazole (AMBBT), has so far only been detected in losartan potassium.

These azido impurities and sartan drug substances are comprised of a similar backbone structure, a tetrazole ring bonded to a phenyl group, which incorporates another phenyl group at its ortho-position (Fig. 1). In the absence of additional information from in vivo studies, it is necessary to ensure that these azido impurities are controlled at or below the Threshold of Toxicological Concern (1.5 µg per person per day, ICH M7).[3] As a result, accurate and reliable quantitation of the azido impurities in sartan drugs is critical.

In this application, we demonstrate a single LCMS method for highly sensitive and accurate analysis of the two azido impurities – AZBT and AMBBT using a Shimadzu LCMS-8060 triple quadrupole mass spectrometer. Full separation of the two azido impurities and the five sartan drug substances was achieved in only 8.5 minutes using a Shim-pack™ Velox SP-C18 column (PN: 227-32003-03).

■ Materials and Methods

Sartan drug substances (olmesartan, losartan, irbesartan, valsartan, and candesartan) were purchased from Sigma-Aldrich. Two azido impurities were obtained from Toronto Research Chemicals. LC-MS-grade solvents (formic acid, methanol, water) were sourced from Honeywell.

AZBT and AMBBT standards preparation

1 mg of each AZBT and AMBBT were accurately weighted and dissolved in 1 mL of methanol to make the initial standard stock solution with a concentration of 1000 µg/mL. The initial standard stock solution was further diluted to 1 µg/mL by adding 100% methanol. A series of calibration standards were prepared from 1 µg/mL stock solution using 90% methanol in water as diluent to obtain the final concentrations of 500, 200, 100, 50, 20, 10, 5, 2, 1, 0.5, 0.2, 0.1, 0.05, 0.02, 0.01, 0.005 ng/mL for both AZBT and AMBBT.

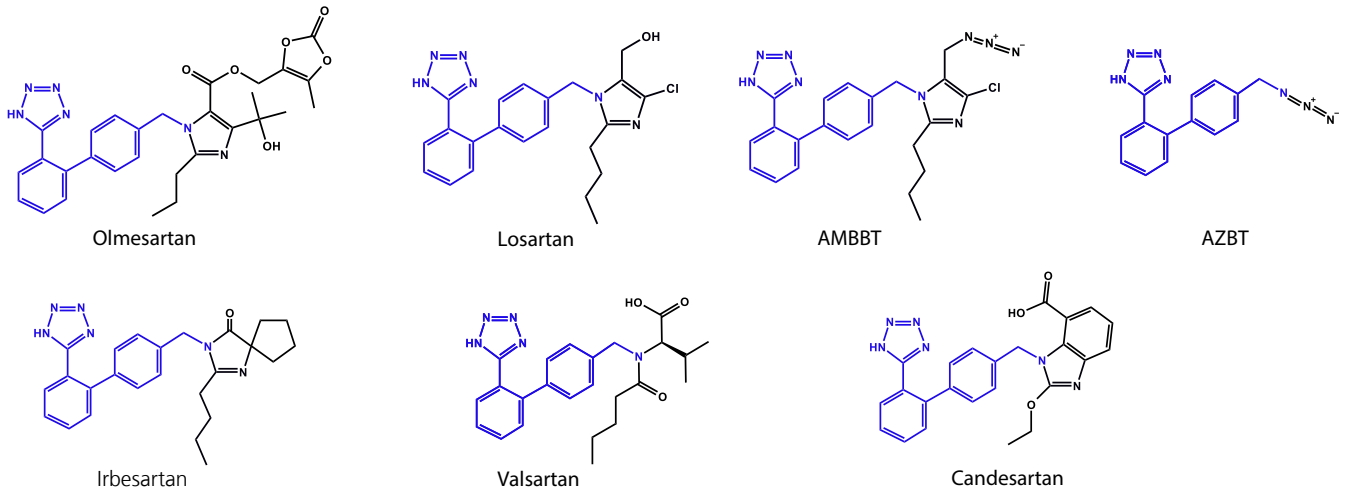

Fig.1 Chemical structures of the five sartan drug substances and the two azido impurities.

Sample preparation

Approximately 5 mg of each sartan drug substances (olmesartan, losartan, irbesartan, valsartan, and candesartan) were weighed and dissolved in 1 mL of the diluent (90% methanol in water). Each sample stock solution was vortexed for 1 minute followed by 10 minutes of sonication to ensure complete dissolution.

Instrumentation parameters

A Shimadzu LCMS-8060 with an SPD-M40 PDA detector was used to provide the highly sensitive and robust analysis. The separation of sartan drugs and azido impurities was achieved in only 8.5 minutes using Shim-pack Velox SP-C18 column which maximizes LC separation performance with core shell technology. A divert valve program was developed in order to send only the 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. Details of instrumentation parameters are shown in Table 1-3.

Table 1 LC-40 conditions

Column	: Shim-pack Velox SP-C18 (100mm x 2.1mm, 2.7µm) [PN: 227-32003-03]
Mobile Phase A	: Water containing 5 mM ammonium formate and 0.1% formic acid
Mobile Phase B	: Methanol
Gradient Program	: 50% B (0 - 1 min) -> 70% B (2 min) -> 98% B (5.3 - 7 min) -> 50% B (7.1 - 8.5 min)
Flow Rate	: 0.4 mL/min
Column Oven Temperature	: 40 ˚C
Autosampler Temperature	: 4 ˚C
Injection Volume	: 5 µL
Stop Time	: 8.5 min
PDA Wavelength Range	: 190 - 800 nm
Divert Valve Program	: Waste (0 - 2.5 min) -> MS (2.5 - 3.2 min) -> Waste (3.2 - 5 min) -> MS (5 - 5.7 min) -> Waste (5.7 - 8.5min)

Table 2 MS conditions

Interface	: ESI
Mode	: MRM
Polarity	: Positive
Nebulizing Gas Flow	: 3.0 L/min
Heating Gas Flow	: 12.0 L/min
Interface Temperature	: 300 ˚C
DL Temperature	: 250 ˚C
Heat Block Temperature	: 400 ˚C
Drying Gas Flow	: 5.0 L/min

Table 3 Detailed MRM settings

Compound	Precursor Ion (*m/z*)	Product Ion (*m/z*)	Dwell Time (ms)	Q1 (V)	Collision Energy (V)	Q3 (V)
AZBT (Quantifier)	278.00	235.15	163.0	-11.0	-10.0	-10.0
AZBT (Qualifier)	278.00	207.10	163.0	-11.0	-16.0	-14.0
AMBBT (Quantifier)	448.15	207.10	163.0	-17.0	-24.0	-21.0
AMBBT (Qualifier)	448.15	405.15	163.0	-17.0	-14.0	-29.0

Injection order

- One injection of null
- One injection of diluent blank (90% methanol in water)
- Six replicate injections of standard solution (5 ng/mL) for system suitability
- Triplicate injections of LOD (0.005 ng/mL)
- Triplicate injections of calibration standards from low to high concentration level (0.01 - 500 ng/mL)
- Triplicate injections of each unspiked sartan sample (1.0 mg/mL)
- For recovery evaluation of AZBT, triplicate injections of spiked samples at three concentration levels for each sartan
- Triplicate injections of unspiked losartan sample (0.1 mg/mL)
- For recovery evaluation of AMBBT, triplicate injections of spiked samples at three concentration levels for losartan
- Four injections of bracket standards (5 ng/mL) throughout the batch
- Four injections of diluent blank during the batch and two injections of the diluent blank at the end

Data analysis. Data was acquired using LabSolutions™ software and analyzed using LabSolutions Insight™ LCMS. Insight features fast data processing and data review, allowing scientists to analyze data more efficiently.

■ Results and Discussion

Chromatographic separation

With the increased resolution power of Shim-pack Velox column, full separation of the five sartan drug substances and two azido impurities was achieved in 8.5 minutes. Fig. 2 illustrates a representative UV chromatogram of the five sartan drugs and its overlapping with the MRM total ion chromatograms (TIC) of AZBT and AMBBT. The blue lines in Fig. 2 show the time points for divert valve switching. The combination of LC chromatographic separation and divert valve program ensured only the two azido impurities would be injected into the highly sensitive mass spectrometer and avoided contamination from heavy sample load.

System suitability and reproducibility

System suitability and reproducibility for both AZBT and AMBBT was evaluated by six replicate injections of the standard solution at 5 ng/mL and four extra bracket injections of the standard solution throughout the batch. Results were summarized in Table 4 (AZBT) and Table 5 (AMBBT). For both impurities, %RSD of the retention time and peak area for all ten injections was less than 3%, which indicates the excellent robustness and reproducibility of the system.

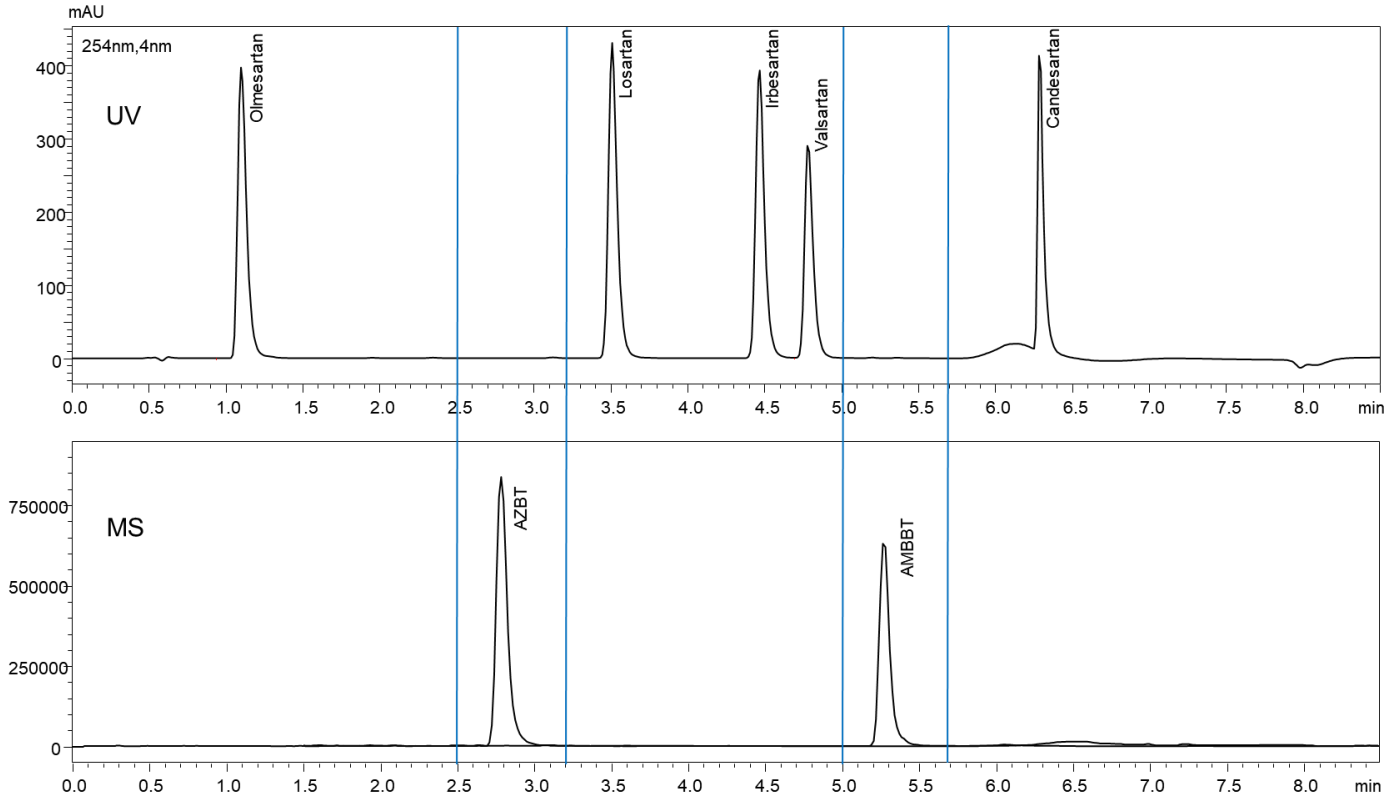

Fig. 2 A representative UV chromatogram of the five sartan drugs and MS MRM total ion chromatogram (TIC) of AZBT and AMBBT. Blue lines show the time points for divert valve switching.

Table 4 System suitability and reproducibility results of AZBT

	Number	RT (min)	Area	Calculated conc. (ng/mL)
Initial replicates	1	2.778	1440283	4.983
	2	2.775	1447854	5.009
	3	2.779	1447695	5.009
	4	2.777	1437430	4.973
	5	2.777	1517842	5.252
	6	2.778	1503493	5.202
Bracket standards	7	2.776	1522246	5.267
	8	2.778	1513057	5.235
	9	2.780	1425233	4.931
	10	2.779	1439822	4.982
	Average	2.778	1469496	5.084
	Std Dev	0.001494	39212.1	0.1357
	%RSD	0.1	2.70	2.70

Table 5 System suitability and reproducibility results of AMBBT

	Number	RT (min)	Area	Calculated conc. (ng/mL)
Initial replicates	1	5.270	1526722	5.053
	2	5.270	1528349	5.059
	3	5.272	1543055	5.107
	4	5.271	1541265	5.101
	5	5.268	1631733	5.401
	6	5.270	1594880	5.279
Bracket standards	7	5.269	1595266	5.280
	8	5.267	1582259	5.237
	9	5.271	1545476	5.115
	10	5.269	1609229	5.326
	Average	5.270	1569823	5.196
	Std Dev	0.001494	37294.9	0.1235
	%RSD	0.03	2.38	2.38

LOD and LOQ. The detection limit (LOD) and quantitation limit (LOQ) data is summarized in Table 6. LOD was determined based on peak area response and signal-to-noise (S/N) ratio of no less than 3.[4] LOQ was determined based on accuracy, reproducibility, and S/N ratio of no less than 10. The accuracy of the data points at LOQ was within 90 - 110% with the %RSD of 9%. Representative chromatograms of the quantifier ions in LOD and LOQ injections were shown in Fig. 3.

Table 6 Summary of LOD and LOQ data

Compound	LOD		LOQ	
	ng/mL	S/N*	ng/mL	S/N*
AZBT	0.005	6.04	0.01	15.16
AMBBT	0.005	6.13	0.01	21.02

*S/N was calculated using rms algorithm with noise range of 0.5 minutes.

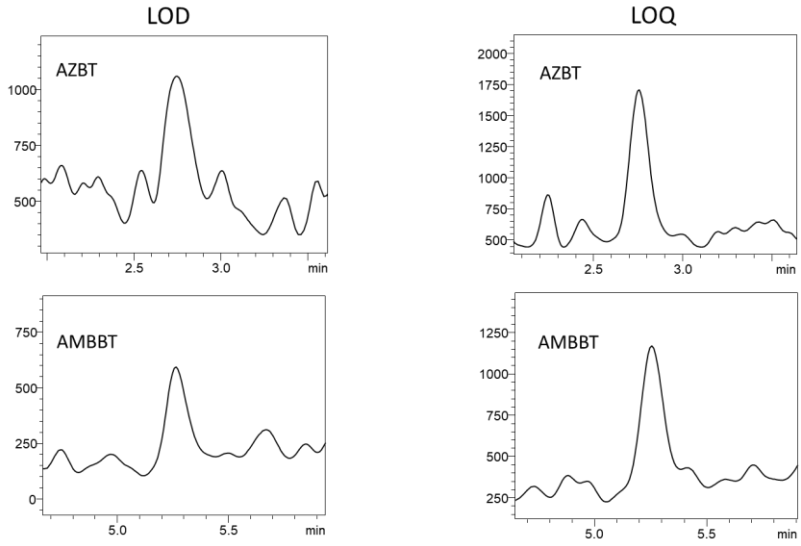

Fig.3 Representative chromatograms of the quantifier ions in LOD and LOQ injections.

Linearity

A linear calibration curve for AZBT was achieved in the concentration range of 0.01 - 500 ng/mL. The calibration range for AMBBT was 0.01 - 200 ng/mL.

Fig. 4 shows the linearity of the azido standards. Excellent linearity with R^2 greater than 0.999 for both compounds was achieved over four orders of magnitude. The accuracy of all calibrators was within the range of 80 - 120%.

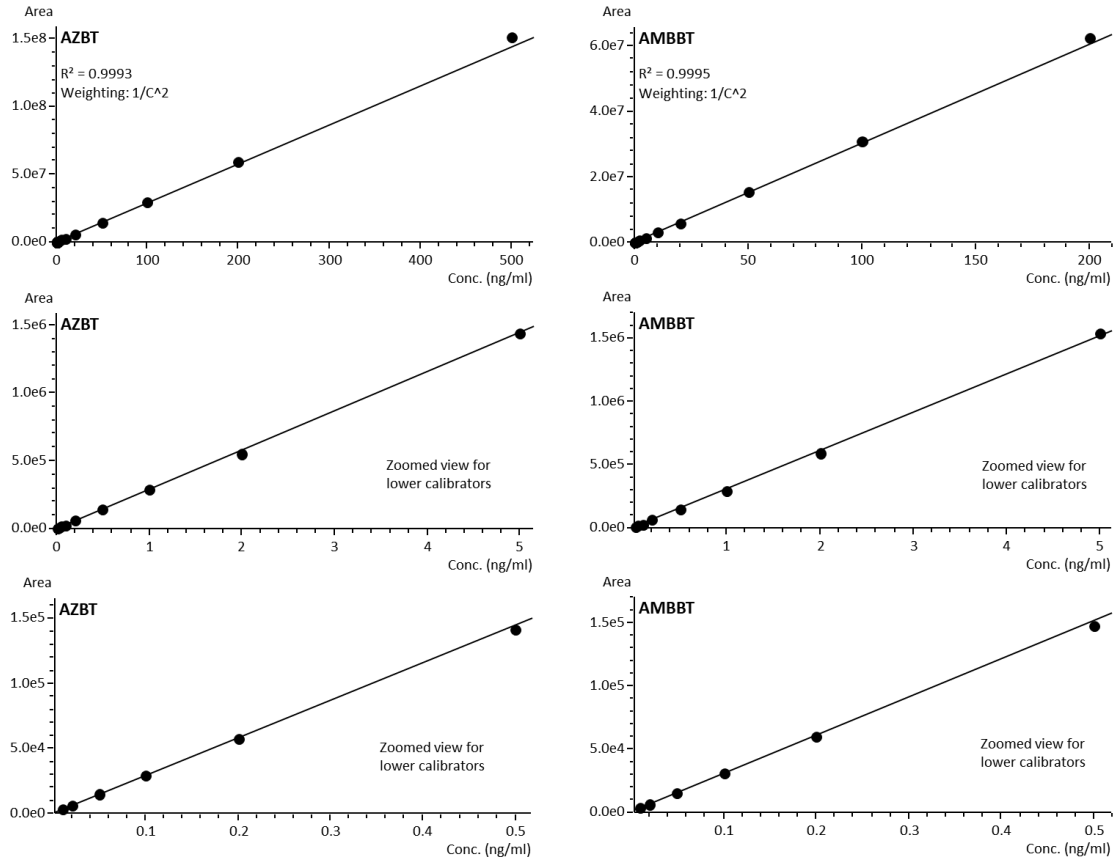

Fig. 4 Calibration curves for AZBT and AMBBT, and the zoomed views for lower calibrators.

Recovery results

The recovery for AZBT was evaluated by spiking three levels of AZBT (0.1, 5.0, 50 ppm) into 1.0 mg/mL of each sartan drug substance except losartan and irbesartan. Losartan and irbesartan were spiked with 0.5 ppm of AZBT for recovery analysis since high concentrations of AZBT already existed (~10 ppm for losartan, ~90 ppm for irbesartan) in the original samples. The recovery for AMBBT was evaluated by spiking three levels of AMBBT (5.0, 50, 500 ppm) into 0.1 mg/mL of losartan drug substance as the amount of AMBBT in the unspiked losartan was high (~1530 ppm).

Recovery was calculated using the equation below. Recovery % observed at each impurity level was within 70 - 130%.[4] The results for the recovery experiments were summarized in Table 7. No carryover was observed in the diluent blank injections during or at the end of the batch.

$$\text{Recovery \%} = \frac{\text{Peak area of spiked sample} - \text{peak area of unspiked sample}}{\text{Peak area of neat azido standard}} \times 100$$

Table 7 Recovery results for AZBT and AMBBT

AZBT Recovery in 1.0 mg/mL Sartan API				
Sartans	Amt. in unspiked sample (ppm)	Spiked amt. (ppm)	Amt. in spiked sample (ppm)	Recovery %
Olmesartan	0.27	0.10	0.38	110
		5.00	5.48	104
		50.00	49.55	98
Losartan	9.41	0.50	10.05	128
		5.00	14.53	102
		50.00	57.83	97
Irbesartan	89.06	0.50	89.47	82
		5.00	95.19	123
		50.00	133.28	88
Valsartan	0.29	0.10	0.40	115
		5.00	5.19	98
		50.00	51.58	103
Candesartan	0.28	0.10	0.41	124
		5.00	5.20	98
		50.00	48.12	96
AMBBT Recovery in 0.1 mg/mL Losartan				
Losartan	1530.22	5.00	1536.54	126
		50.00	1565.98	71
		500.00	1945.86	83

■ Conclusion

In this application, a single LCMS method was successfully developed for the analysis of AZBT and AMBBT in five sartan drug substances (olmesartan, losartan, irbesartan, valsartan, and candesartan). Full separation of all sartans and azido impurities was achieved in only 8.5 minutes using the Shim-pack Velox SP-C18 column. A divert valve was used to deliver azido impurities into the mass spectrometer and protect the detector from contamination from sartan drugs.

A linear relationship was obtained in a wide calibration range for AZBT (0.01 – 500 ng/mL) and AMBBT (0.01 – 200 ng/mL) with R^2 over 0.999. Recovery experiments were performed, and the results were all within 70 - 130%.

This application demonstrates the sensitivity and reproducibility of the Shimadzu LCMS-8060 in the detection of azido impurities in sartan drug substances.

■ References

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