SHIMADZU

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Quantitative Analysis of Azido Impurities in Five Sartan Drug Substances using a Triple Quadrupole Mass Spectrometer

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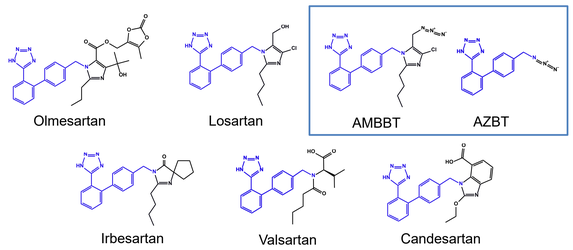
1. Overview

A single LCMS method was successfully developed for the analysis of azido impurities in five sartan drug substances.

2. Introduction

Sartans belong to a class of medicines used for patients with hypertension (high blood pressure) and those with certain heart or kidney diseases. Over the past few years, sartan medications have been recalled due to concerns about azido impurities, a probable carcinogen. 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). As a result, accurate and reliable quantitation of the azido impurities in sartan drugs is critical.



3. Methods

In this study, two azido impurities, (5-(4' (azidomethyl)-[1,1'-biphenyl]-2-yl)-1H-tetrazole, also known as azidomethyl-biphenyl-tetrazole (AZBT), and losartan azido impurity, 5-[4'-[(5-(azidomethyl)-2-butyl-4-chloro-1H-imidazol-1-yl)methyl]-[1,1'-biphenyl]2-yl]-1H-tetrazole (AMBBT) were measured in five sartan drug substances (olmesartan, losartan, irbesartan, valsartan, and candesartan). 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 a Shim-pack Velox SP-C18 column. A divert valve program was developed in order to send only the azido impurities into the mass spectrometer for highly sensitive detection while deliver the high amount of drug substances to waste in order to avoid mass spectrometer contamination.

4. Results

Chromatographic Separation. Full separation of the five sartan drug substances and two azido impurities was achieved in 8.5 minutes. Figure 1 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 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.

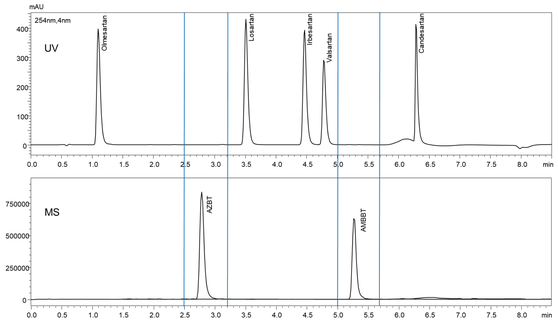


Figure 1: A representative UV chromatogram of the five sartan drugs and its overlapping with MRM total ion chromatograms (TIC) of AZBT and AMBBT. Blue lines show the time points for divert valve switching.

Table 1. System suitability and reproducibility results of AZBT and AMBBT

	Number	AZBT RT (min)	AZBT Conc. (ng/mL)	AMBBT RT (min)	AMBBT Conc. (ng/mL)
Initial replicates	1	2.778	4.983	5.270	5.053
	2	2.775	5.009	5.270	5.059
	3	2.779	5.009	5.272	5.107
	4	2.777	4.973	5.271	5.101
	5	2.777	5.252	5.268	5.401
	6	2.778	5.202	5.270	5.279
Bracket standards	7	2.776	5.267	5.269	5.280
	8	2.778	5.235	5.267	5.237
	9	2.780	4.931	5.271	5.115
	10	2.779	4.982	5.269	5.326
	Avg.	2.778	5.084	5.270	5.196
	%RSD	0.1	2.70	0.03	2.38

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 1. 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.

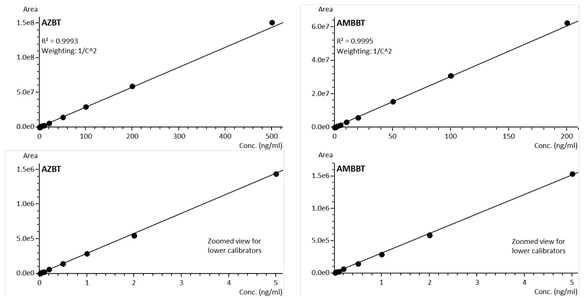
LOD and LOQ. The detection limit (LOD) and quantitation limit (LOQ) data was summarized in Table 2. LOD was determined based on peak area response and signal-to-noise (S/N) ratio to be no less than 3. LOQ was determined based on accuracy, reproducibility and S/N ratio to be no less than 10. The accuracy of the data points at LOQ was within 90–110% with the %RSD of 9%.

Table 2: 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 minute.

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 as the signal was saturated at 500 ng/mL. Figure 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%.



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%. 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.

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

5. Conclusions

- 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.
- Divert valve was used to deliver azido impurities into mass spectrometer and protect the detector from the contamination of 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 Shimadzu LCMS-8060 in the detection of azido impurities in sartan drug substances.