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Separation and Quantification of an Azidomethyl-biphenyl-tetrazole (AZBT) Impurity in five Sartan Drug Substances using LCMS-8045

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1. Overview

In October 2020, pharmaceutical companies and regulatory bodies around the world found traces of Azidomethyl-biphenyl-tetrazole (AZBT) impurity in batches of the Active Pharmaceutical Ingredient (API) i.e., Irbesartan. AZBT is a compound that can form during the manufacture of the API in some sartan drugs. It is known to damage DNA, and as a result, long-term exposure over years may increase an individual's risk of developing cancer. The risk posed by this AZBT impurity at the levels detected in sartan medicines to date is very low. However, such contamination is considered unacceptable for a medicine[1].

2. Introduction

The sartan group of medicinal products include preparations containing the active substances Valsartan, Losartan, Olmesartan, Candesartan and Irbesartan, as well as several other active substances. These medicinal products are also known as angiotensin II receptor antagonists. Sartans have antihypertensive and vasodilatory effects and are prescribed for the treatment of high blood pressure, heart failure or kidney disorders in order to prevent heart attacks or strokes.[2]

This AZBT is an intermediate in the synthesis pathway for each of the API and comprised of a similar backbone structure. Sartan drugs contain a tetrazole ring bonded to a phenyl group, which incorporates another phenyl group at its ortho-position. The differences between the compounds is at the para-position of the second phenyl ring (Figure 1); hence the need for a suitable analytical technique to accurately detect and quantify the AZBT impurity from sartan APIs. Considering the cost and challenges such as structural similarities, it is imperative to have a sensitive, reliable & accurate method for determination of AZBT impurity in sartan drugs.

This poster describes a validated LC-MS/MS method with optimized sample preparation procedure to determine AZBT in Valsartan, Losartan, Olmesartan, Irbesartan and Candesartan..
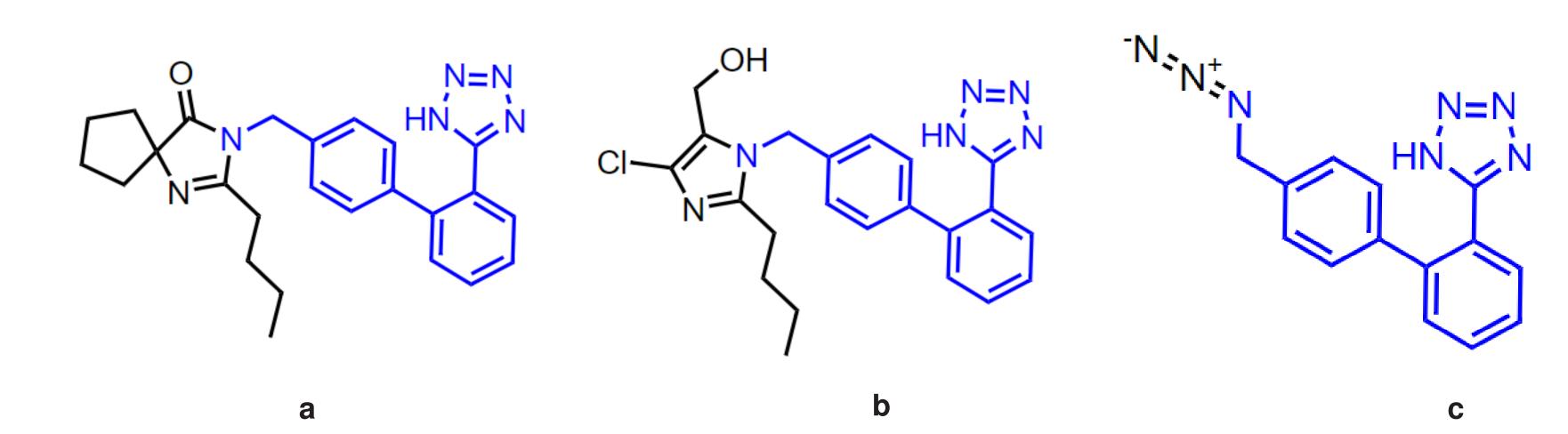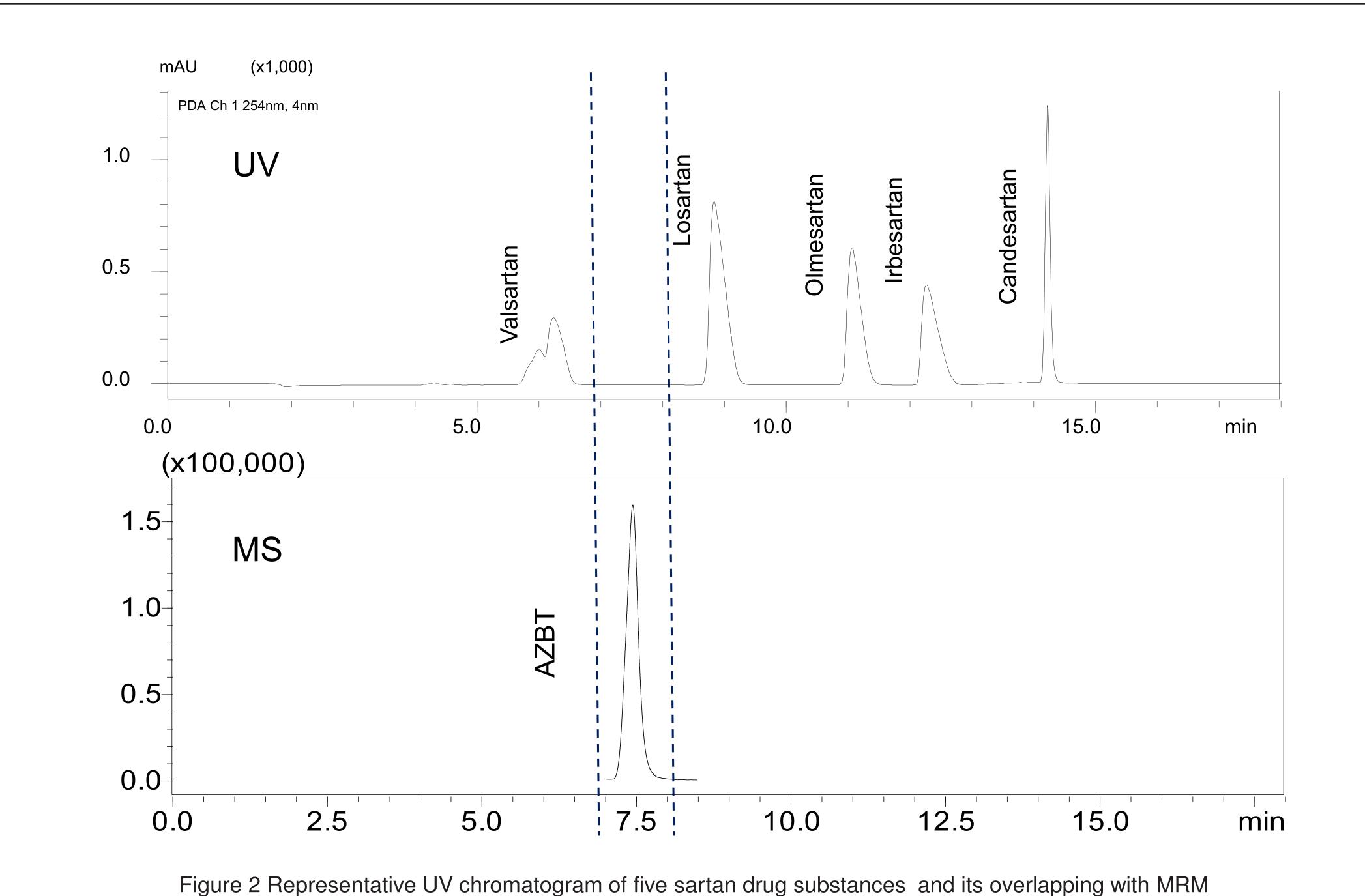

Figure1: Structure of representative sartan drugs like Irbesartan (a), Losartan (b) & AZBT (c)

3. Materials and methods

3-1. Method development

Locally procured AZBT impurity standard was used for method development. AZBT standard was prepared in water:methanol (10:90) diluent and analysed in scan mode. To remove interference from matrix and possible contamination of mass spectrometer due to high concentration of API a suitable liquid chromatography method was developed (Table 1) along with divert valve function (Figure 2). For mass spectrometric development, steps such as precursor ion selection & Multi Reaction Monitoring (MRM) optimization at different collision energies were performed to obtain MRMs and their optimum collision energies. (Table 2)

Shimadzu LCMS-8045, (Figure 3) sets a new benchmark in triple quadrupole technology with an unsurpassed sensitivity (UFsensitivity), ultra fast scanning speed of 30,000 u/sec (UFscanning) and polarity switching speed of 5 msec (UFswitching). This system ensures highest quality of data, with very high degree of reliability.

Figure 2 Representative UV chromatogram of five sartan drug substances and its overlapping with MRM chromatogram of AZBT

Figure 3: Nexera XS with LCMS-8045 system

3-2. Analytical conditions

Table 1:. Instrument parameters

UHPLC condition (Nexera XS system)	
Column	Shim-pack Scepter Phenyl (150 mm x 4.6 mm, 3 micron) (P/N :227-31067-05)
Mobile phase	A: 10mM Ammonium formate in water B: Methanol
Flow rate	0.4 mL/min
Gradient program (B%)	0-8 min → 60(%); 8-9 min → 60-95 (%); 9-15 min → 95 (%); 15-15.1 min → 95-60 (%); 18 min → Stop.
Injection vol.	5 µL
Column temperature	45°C
Diluent	Water:methanol (10:90)

Table 2. MS acquisition parameters

MS Parameters (LCMS-8045)	
MS interface	ESI
Nebulizing gas flow	3 L/min
Drying gas flow	5 L/min
Heating gas flow	10 L/min
Desolvation line temp.	250°C
Heating block	300°C
Interface	350°C

Acquisition mode	Polarity	MRM quantifier	MRM qualifier
MRM	Positive	278>235	278>207

3-3. Sample preparation

The 0.030 g of sartan drug substance was taken into 100 mL standard volumetric flask, then 50 mL of diluent was added. The sample was sonicated till it dissolved and made up to the volume. The clear solution was filtered using 0.45-µm nylon syringe filter and considered for the LC-MS analysis. The overall sample concentration was 0.3 mg/mL. The same procedure was employed for the preparation of all the five sartan drug substances. Recovery samples were prepared by spiking LOQ level of AZBT impurity into the sample

4. Results

4-1. Linearity of the AZBT impurity

For quantitation, a seven-point calibration curve for AZBT impurity ranging from 0.09* to 8.0* ppb was analyzed & plotted. The results obtained for linearity are summarized in table 3.

* As such concentration

The figure 4 depicts the calibration curve, overlay of linearity standards & LOQ solution chromatograms for AZBT impurity.
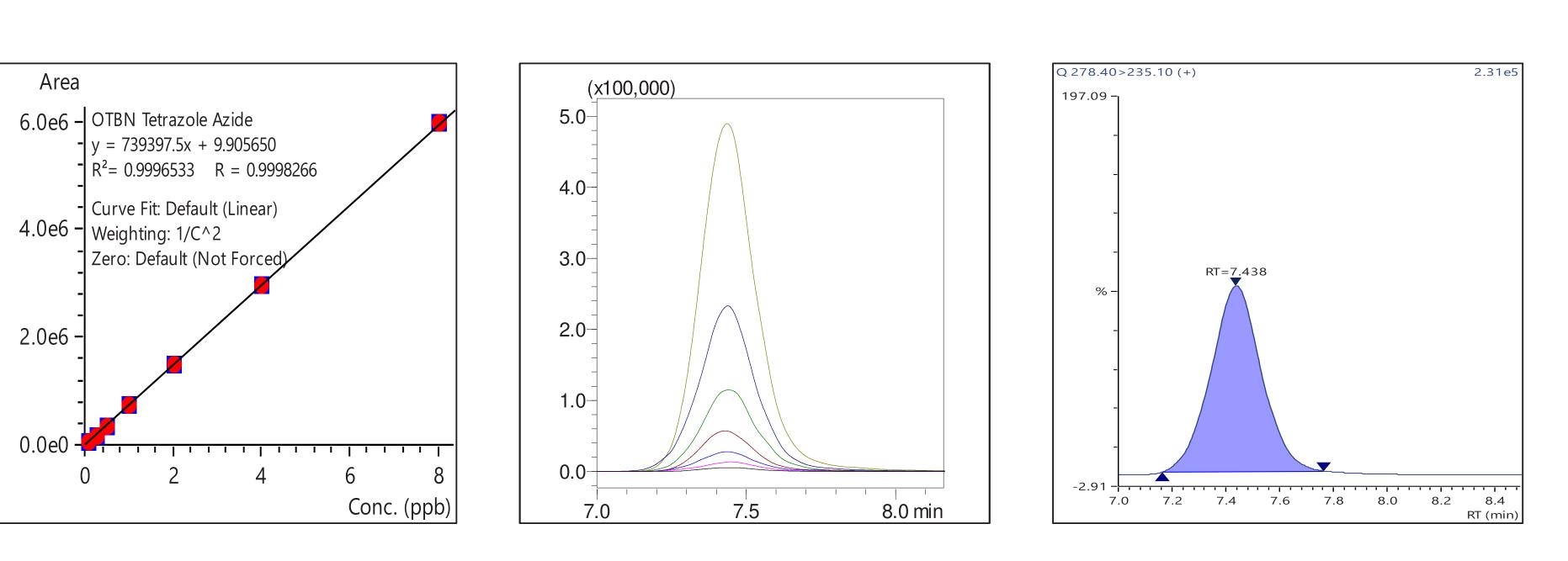

Figure 4 Calibration curve, overlay of linearity standards & LOQ solution chromatogram for AZBT

Table 3. Summary of linearity & LOQ results

Compound	r²	LOQ		
		Concentration	%RSD (n=6)	S/N
AZBT	0.999	300 ppb*	2.5	201

* Concentration w.r.t. sample of 0.3 mg/mL.

4-2. Recovery in samples

All sartan API samples were spiked with 300 ppb AZBT standard and were analyzed using the same analytical conditions. The results obtained for recovery samples are summarized in table 4.

Table 4. % Recovery at LOQ

Sartans	Amt. found in sample (ppb)	Spiked amt. (ppb)#	Amt. found in spiked sample (ppb)	% Recovery
Valsartan	561		889	103
Losartan	42		390	114
Olmesartan	BLOQ	300	316	105
Irbesartan	295		641	108
Candesartan	BLOQ		359	120

Spiked at LOQ level i.e. 0.09 ppb which is equivalent to the 300 ppb w.r.t. sample concentration of 0.3 mg/mL.
BLOQ : Below limit of quantitation

5. Conclusion

- A single LCMS method for the quantification of Azidomethyl-biphenyl-tetrazole (AZBT) impurity in five sartan drug substances has been successfully developed on the Shimadzu LCMS-8045 system.

- Seven levels of linearity was performed for the AZBT impurity and correlation coefficient was greater than 0.995.

- The repeatability (n=6) at LOQ level was found to be less than 5% RSD.

- Recovery analysis was performed at LOQ level, and it matched to the acceptance criteria between 80 to 120 %.

6. References

[1] TGA investigation - low levels of contamination with azidomethyl-biphenyltetrazole (AZBT), 20 August 2021
[2] Monitoring of sartan medicines stepped up: traces of a new foreign substance detected, 01 July 2021