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Pharma

A validated LC-HRAM-MS method for the rapid and confident determination of azido (AZBT) impurity in sartan drug products

Authors

Varun Khali[1], Sachin Pandey[1], Manoj Kushwaha[1], Ramiz M. R. Azad[2], Deepti Bhandarkar[3], Saravanan Kumar[4], Biswajayee A. Patra[1], Sven Hackbusch[5], Jon Bardsley[6], Aaron Lamb[6]

Thermo Fisher Scientific, India Private Limited, Customer Solution Centre, [1]Mumbai, India; [2]Ghaziabad, India; [3]Ahmedabad, India; [4]Bangalore, India

Thermo Fisher Scientific, [5]San Jose, CA, USA; [6]Hemel Hempstead, UK

Keywords

Orbitrap Exploris 120 MS, LC-HRAM, LC-HRMS, Vanquish Flex UHPLC, liquid chromatography, high resolution t-MS^2, AZBT, candesartan, irbesartan, losartan, olmesartan, telmisartan, valsartan

Key benefits

- A single method capable of selectively quantifying AZBT impurity in six different sartan products
- High-resolution, accurate mass (HRAM) mass spectrometry allows separation of closely related interferences
- A validated method in line with regulatory information and requirements mentioned in EDQM,[1] USFDA (Q2B),[2] EMEA,[3] and ICH M7[4] guidelines

Goal

The aim of this study was to develop and report the quantitative capabilities of the Thermo Scientific™ Orbitrap Exploris™ 120 mass spectrometer in combination with liquid chromatography for selective quantitation of azidomethyl-biphenyl-tetrazole (AZBT) in six different sartan drug products on a Thermo Scientific™ Accucore™ Biphenyl column using a single LC-HRAM-MS method.

Introduction

Sartans, which are also called angiotensin II receptor blockers (ARBs), are medicines that are used to treat high blood pressure (also known as hypertension). In 2018, the U.S. Food and Drug Administration (U.S. FDA) and the European Medicines Agency (EMA) initiated investigations of nitrosamine impurities in certain sartan-containing

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active pharmaceutical ingredients (APIs), such as valsartan, candesartan, irbesartan, losartan, and olmesartan. Following this observation, in October 2020 pharmaceutical companies and Swissmedic found traces of impurity in irbesartan drug substance that was not a nitrosamine. This impurity was found to be azidomethyl-biphenyl-tetrazole (AZBT) and it was observed to have mutagenic properties.

This azido impurity is a compound that can form during the manufacturing of tetrazole-containing sartan drug substances. However, the level of this impurity needs to be controlled and measured in the finished drug products due to its mutagenic effect.

As per ICH M7,[4] a Threshold of Toxicological Concern (TTC)-based acceptable intake of a mutagenic impurity of 1.5 µg per person per day is associated with a negligible risk (theoretical excess cancer risk of <1 in 100,000 over a lifetime of exposure) and can in general be used for most pharmaceuticals as a default to derive an acceptable limit for control. Therefore, developing a highly specific, selective, as well as sensitive method to quantify this azido impurity level(s) at or below TTC is of prime importance for the pharmaceutical manufacturers.

In this study, an LC-HRAM Orbitrap MS-based method was developed that can selectively and accurately quantify AZBT impurity in six different sartan drug products—candesartan, irbesartan, losartan, olmesartan, telmisartan, and valsartan (Figure 1). In such cases where trace level impurities are quantified in the presence of drug substances or drug product samples, the quantity of sample is generally in milligrams and that is too high of a sample load to be considered suitable for MS. Therefore, the first step is to separate the peaks of the Impurity and API sample. Then a suitable time segment is identified in which the impurity elutes. For this time segment, the LC flow is delivered to the MS, and for the rest of the time, it is diverted away from the MS. Failing to do so may result in contamination of the mass spectrometer, which may lead to noisy baselines, reduced sensitivity, poor repeatability, poor robustness, etc. Certain LC-MS/MS methods[5-8] are available in literature for determination of AZBT in some of the sartans. However, in this application note, an LC-HRAM-MS method was developed in which the chromatographic separation of all six sartan APIs and AZBT is achieved using Thermo Scientific™ Accucore™ Biphenyl chemistry and a divert valve, placed in the appropriate position to avoid contamination of the mass spectrometer and obtain robust performance. Furthermore, highly selective, accurate, and sensitive quantification of AZBT impurity is achieved by using an Orbitrap Exploris 120 mass spectrometer, which provides consistently accurate data and a design made for operational simplicity, setting a new standard in instrument productivity and ruggedness. The resolution of 120,000 (FWHM) at *m/z* 200 of the Orbitrap Exploris 120 mass spectrometer enables resolving target compounds from matrix interferences and accurate quantitation even in complex samples such as drug products.

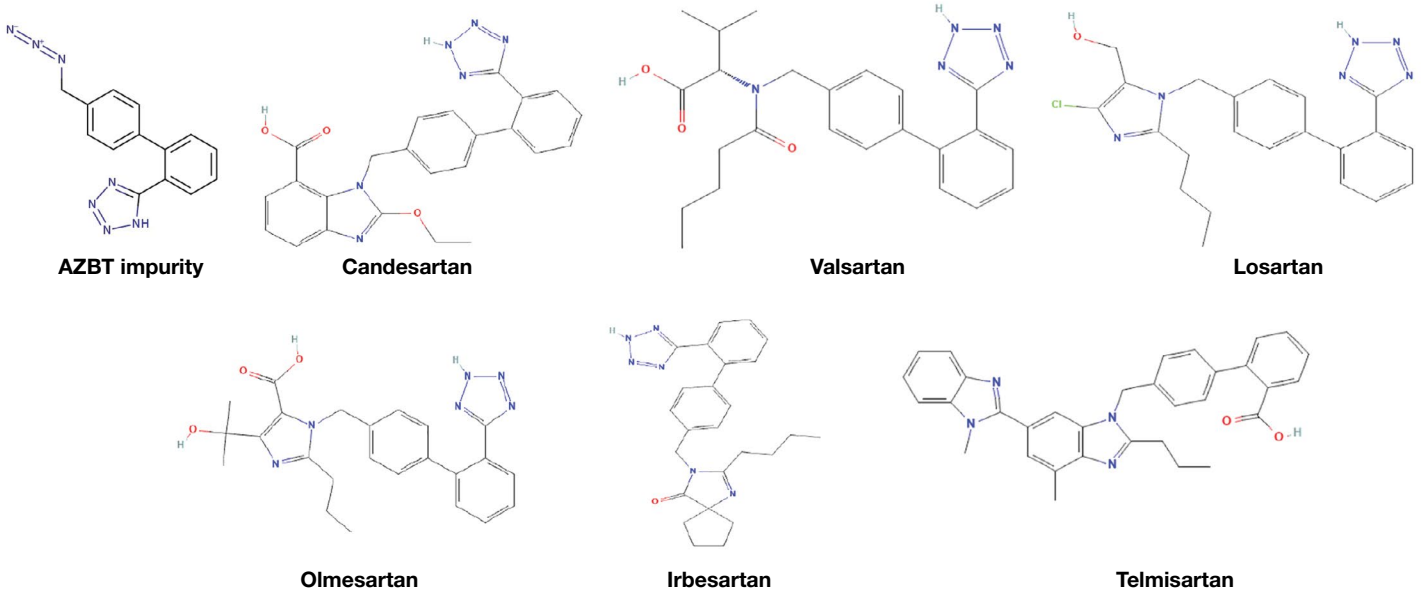

Figure 1. Chemical structures of AZBT impurity and six sartans analyzed in this study

Experimental

Sample preparation

Diluent solution and blank preparation

80 mL of methanol and 20 mL of water were mixed and used as the diluent solution and blank.

Standard stock solution (100 µg/mL)

An appropriate amount of AZBT reference standard was weighed and dissolved in methanol to achieve a concentration of 100 µg/mL.

Preparation of system suitability standard (5 ng/mL)

10 µL of standard stock solution was transferred into a 20 mL volumetric flask and diluted to volume using diluent solution to prepare the intermediate dilution. A 1 mL aliquot volume of the intermediate dilution was transferred into a 10 mL volumetric flask and diluted to volume with diluent solution. This standard solution can be used for system suitability experiment as well as recovery evaluation at the mid concentration level.

Preparation of linearity standards, limits of detection (LOD) and limits of quantitation (LOQ) levels

An intermediate dilution of 1,000 ng/mL was prepared by transferring 0.1 mL of standard stock solution (100 µg/mL) into a 10 mL volumetric flask and making up the volume to the mark with diluent solution. From this intermediate dilution, a suitable volume was serially diluted to achieve seven linearity standards of concentrations 0.25 (LOQ), 0.5, 1.25, 5, 10, 20, and 40 ng/mL. LOQ was further diluted appropriately to prepare LOD of 0.025 ng/mL.

Sample preparation procedure

Approximately 5 to 10 tablets of the sartan drug product were ground into powder and mixed properly. An appropriate weight corresponding to 5 mg of active content was weighed and transferred into a 15 mL centrifuge tube. The sample was made up with 5 mL of diluent solution, vortexed briefly, and then sonicated for 30 minutes in an ultrasonic bath. The samples were vortexed for 1 minute and centrifuged at 4,500 rpm and 5 °C for 15 minutes. The clear supernatant was transferred into autosampler vials for analysis.

Instrumentation

- Thermo Scientific™ Vanquish™ Flex Binary UHPLC system equipped with a temperature-controlled autosampler and column compartment
- Thermo Scientific™ Orbitrap Exploris™ 120 mass spectrometer with the Thermo Scientific™ OptaMax™ NG ion source and the heated electrospray ionization (H-ESI II) probe

Consumables/reagents

- Reference standards procured from Cleanchem Laboratories
  - 5-(4'-(azidomethyl)-[1,1'-biphenyl]-2-yl)-1H-tetrazole (AZBT)
- Fisher Scientific™ Formic acid, Optima™ LC/MS grade (Fisher Scientific P/N A117-50 or equivalent)
- Fisher Scientific™ Methanol, Optima™ LC/MS grade (Fisher Scientific P/N A456-4 or equivalent)
- Fisher Scientific™ Water, Optima™ LC/MS grade (Fisher Scientific P/N AAB-W6-4 or equivalent)
- Thermo Scientific™ Nunc™ 15mL extraction/ conical sterile polypropylene centrifuge tubes (P/N 339652)
- Thermo Scientific™ Chromacol™ GOLD HPLC vials (2-SVG)
- Thermo Scientific™ Accucore™ Biphenyl column, 100 × 2.1 mm, 2.6 µm (P/N 17826-102130)

LC-HRAM-MS conditions

A Vanquish Flex binary UHPLC system coupled to an Orbitrap Exploris 120 high-resolution mass spectrometer was used for the data acquisition. UHPLC configurations and parameters are listed in Table 1. The mass spectrometer configuration and acquisition and data processing parameters are listed in Tables 2 and 3. Divert valve settings are given in Table 4. A targeted-MS^2 (t-MS^2) fragmentation-based acquisition mode provides more selectivity because high-resolution product ion scans are attained only for target ions of interest based on a user defined target list, irrespective of any other species contained in the sample, such as complex sample matrices. Thus, a t-MS^2 acquisition method was employed for this analysis, which offered excellent selectivity and sensitivity for the analysis of AZBT from different sartan drug products.

Table 1. LC conditions

Parameter	Value
HPLC column	Accucore Biphenyl 100 x 2.1 mm, 2.6 µm
Mobile phase	A: 0.1 % formic acid in water B: 0.1 % formic acid in methanol
Flow rate	0.40 mL min^{-1}
Gradient	Time (min) % B 0.0 55 3.0 55 4.0 60 6.0 90 14.0 90 14.2 55 17.0 55
Column temp.	35 °C, Still Air
Injection volume	5 µL
Autosampler temp.	10 °C
Needle wash	Methanol:water (80:20 v/v)
Static mixer volume	150 µL
UV wavelength (for sartans)	224 nm, data collection rate 5 Hz, response time 1.0 s

Table 2. Ion source settings

Parameter	Value
Ion source type and probe	OptaMax NG ion source with H-ESI II probe
Polarity	Positive
Positive voltage	2,500 V
Sheath gas flow rate	50 arbitrary units
Aux gas flow rate	10 arbitrary units
Sweep gas flow rate	0 arbitrary units
Ion transfer tube temp.	275 °C
Vaporizer temp.	375 °C

Table 3. MS acquisition and data extraction parameters

Parameter	Value
Precursor ion *(m/z)*	278.11490
RF Lens (%)	70
Q1 resolution *(m/z)*	1.0
Source fragmentation	Off
Orbitrap resolution	120,000
Collision energy mode	Fixed
Collision energy type	Absolute
Collision energy (V)	5
Scan range mode	Auto
Maximum injection time mode	Auto
m/z to be extracted	235.09782
Mass tolerance (ppm)	5
Internal calibration	Easy IC enabled

Table 4. Divert valve settings

Time (min)	Position	Remarks
0	1-6	Diverted to waste
2.4	1-2	MS
5.2	1-6	Diverted to waste

Injection order

- One injection of diluent blank
- Six replicate injections of standard solution (5 ng/mL) for system suitability
- Three replicate injections of LOD (0.025 ng/mL)
- Six replicate injections of LOQ (0.25 ng/mL)
- One injection each of linearity standard from low to high concentration level
- All as such (un-spiked) drug tablet samples in triplicate
- For spiked sample recovery evaluation: six replicate injections of neat standards at low, mid, and high concentrations along with three replicates of spiked samples at three levels i.e., low, mid, and high concentrations
- Suitable number of bracketing standards and diluent blank injections throughout the sequence

Note: Samples of each sartan drug product (i.e., candesartan, irbesartan, losartan, olmesartan, telmisartan, and valsartan) were prepared separately.

System suitability requirements

- % RSD of peak area for AZBT impurity for the first six replicate injections of standard solution should below 5%.
- The cumulative % RSD of the peak area should be below 10%. (Cumulative % RSD of the peak area is calculated by combining the initial six replicate injections of the standard solution and all subsequent bracketing standard).

Calculation of AZBT content in drug products[5]

$$\text{Amount of AZBT in the sample (µg/g)} = \frac{C \times V}{M \times 1{,}000}$$

Where,

C: the concentration of AZBT in the sample solution calculated by the standard calibration curve (ng/mL)

V: the final make-up volume of the sample (mL)

M: the weight of the sample (g)

Calculation of recovery percentage[3]

Recovery is calculated by comparing peak area responses of spiked and un-spiked samples against neat standards of AZBT. The following formula is used to calculate recovery:

$$\text{\% Recovery of AZBT} = \frac{\text{Peak area response in spiked sample - Peak area response in unspiked sample}}{\text{Average peak area response of 6 replicates of neat spiking standard}} \times 100$$

% Recovery observed at low, mid, and high concentration levels should be within 70–130%.

Data analysis

Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) version 7.2.10 was used to perform data acquisition, data processing, and reporting of all results. The *m/z* peak extraction was attained within a mass tolerance of 5 ppm in the current study.

Results and discussion

In this study, LOD, LOQ, linearity, reproducibility, and recovery evaluations were performed, and the data were found to be within acceptance criteria. Analysis was done by applying a targeted-MS^2 method on the Orbitrap Exploris 120 LC-HRAM system using the H-ESI ionization probe.

System suitability

System suitability was performed by six replicate injections of standard solution at 5 ng/mL to evaluate the performance of the LC-HRAM-MS instrument. Table 5 shows the results of % RSD (reproducibility) for retention time and peak area. It can be observed that % RSD for peak area is 0.85%, which is well within the acceptance criteria of 5%. Similarly, Table 6 shows the results of cumulative % RSD (reproducibility) with bracketing standards for retention time and peak area. Cumulative % RSD of peak area including the bracketing standard is 2.5%, which is also well within the acceptance criteria of 10%. Overall, results indicate the excellent repeatability offered by developed LC-HRAM-MS method.

LOD, LOQ, repeatability, and linearity

Details of quantitative performance parameters such as LOD, LOQ, and repeatability are given below. LOD and LOQ values were established based on peak area repeatability (less than 20% at the LOQ level), % deviation (within ± 20% at the LOQ level; derived from the linearity plot) and signal-to-noise ratio (S/N) (more than 3:1 for the LOD level and more than 10:1 for the LOQ level).

Representative chromatograms of diluent blank, LOD, and LOQ are shown in Figure 2. There was no interfering peak observed at the retention time of AZBT, which shows the selectivity of the t-MS^2 method.

Table 5. System suitability results

Inj #	Injection name	RT (min)	Peak area (counts * s)
3	Standard-1	4.55	542132
4	Standard-2	4.56	540715
5	Standard-3	4.55	535954
6	Standard-4	4.56	535837
7	Standard-5	4.55	535170
8	Standard-6	4.55	529359
Average		**4.6**	**536528**
%RSD		**0.01**	**0.85**

Table 6. System suitability results – reproducibility with bracketing standards

Inj #	Injection name	RT (min)	Peak area (counts * s)
3	Standard-1	4.55	542132
4	Standard-2	4.56	540715
5	Standard-3	4.55	535954
6	Standard-4	4.56	535837
7	Standard-5	4.55	535170
8	Standard-6	4.55	529359
9	Bracket standard 1	4.55	531499
10	Bracket standard 2	4.51	553090
11	Bracket standard 3	4.51	568656
12	Bracket standard 4	4.51	558614
13	Bracket standard 5	4.50	564658
Average		**4.5**	**545062**
% RSD		**0.55**	**2.5**

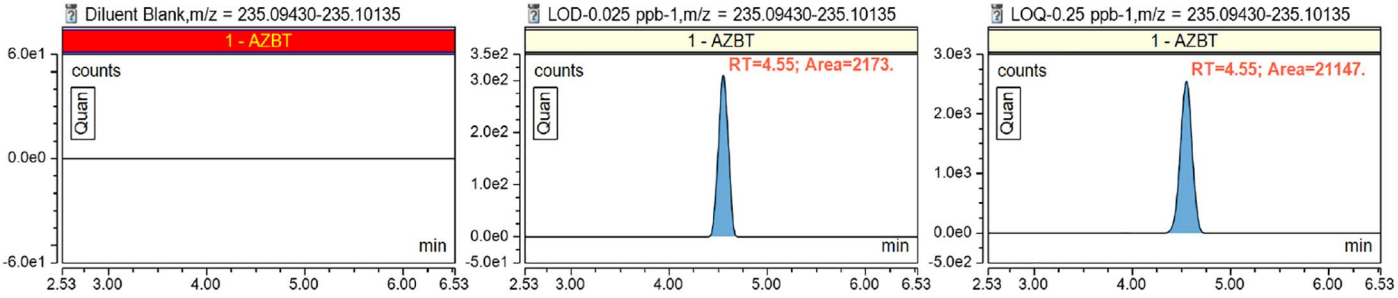

Figure 2. Chromatograms of diluent blank (left), LOD = 0.025 ng/mL (middle), LOQ = 0.25 ng/mL (right)

The data corresponding to the LOD and LOQ is summarized in Table 7. The % RSD for peak area at the LOQ level is less than 3%, indicating good repeatability at this concentration level. Furthermore, the S/N was determined by the "peak to peak" method, and one of the diluent injections was used for the noise calculation. Multiple of time span option was selected for the noise calculation with default parameters in the software for the S/N calculation. It was observed that S/N of >25:1 was obtained for the 0.025 ng/mL concentration, whereas S/N of >240:1 was obtained for the 0.25 ng/mL level, exceeding the criteria for LOD and LOQ, respectively.

Linearity

Linearity was determined by injection of low to high calibration standards of the concentration range, i.e., 0.25 ng/mL to 40 ng/mL. The obtained R^2 value of 0.9992 demonstrates the excellent linear response throughout the concentration range. Figure 3 shows the linearity of the AZBT standard.

Table 7. LOQ reproducibility and S/N at LOD and LOQ levels

Inj #	Injection name	RT (min)	Area (counts * s)	S/N
10	LOD-0.025 ppb-1	4.55	2173	31.3
11	LOD-0.025 ppb-2	4.55	1949	29.0
12	LOD-0.025 ppb-3	4.53	2077	27.2
40	LOQ-0.25 ppb-1	4.55	21147	258.0
41	LOQ-0.25 ppb-2	4.55	21202	256.1
42	LOQ-0.25 ppb-3	4.55	20387	244.8
43	LOQ-0.25 ppb-4	4.54	21504	259.7
44	LOQ-0.25 ppb-5	4.54	20037	238.6
45	LOQ-0.25 ppb-6	4.55	20612	242.0
	Average	**4.5**	**20815**	
	%RSD	**0.12**	**2.7**	

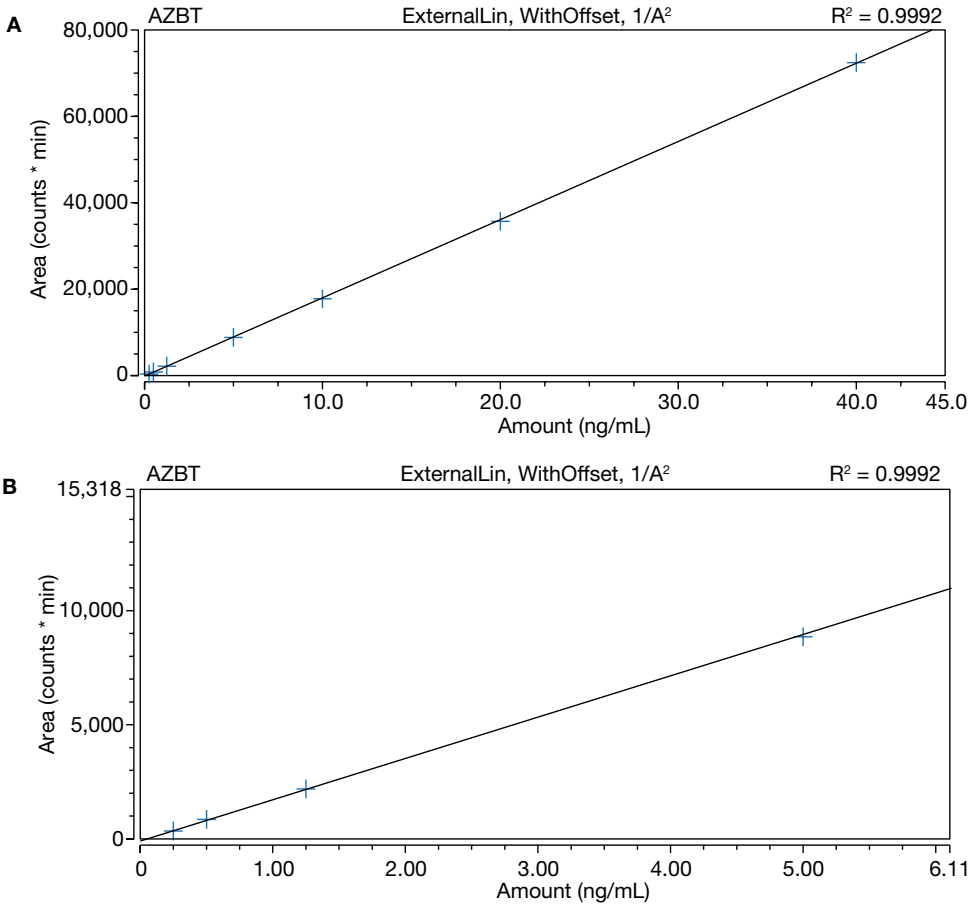

Figure 3. (A) Calibration plot of AZBT from 0.25 ng/mL to 40 ng/mL (un-zoomed) and (B) zoomed calibration plot of AZBT

The results of linearity plot are tabulated in Table 8. It is observed that % deviation was within the range of ± 5% for all the linearity levels including 0.25 ng/mL.

Chromatograms of calibration standard 1 (0.25 ng/mL) and calibration standard 7 (40 ng/mL) are shown in Figure 4.

From the above results, it is observed that S/N obtained for the 0.025 ng/mL level is >25:1 (acceptance criteria is ≥3:1). The % RSD for peak area obtained at 0.25 ng/mL is 2.69% (acceptance criteria is less than 20%), S/N is >240:1 (acceptance criteria is ≥10:1), and % deviation at 0.25 ng/mL is -2.48% (acceptance criteria is within ± 20%).

Depending upon the acceptable intake of 1.5 µg/day for genotoxic impurity as per ICH M7 guidelines[4] and 300 mg as the maximum daily dose of sartans (that is, maximum in the case of the six sartans evaluated here), the standard limit is calculated to be 5 ppm. Based on this calculation, from the sample preparation employed (1 mg/mL equivalent of drug substance) and from the obtained results, it can be concluded that LOD and LOQ concentrations with respect to the drug substance for AZBT analysis are as follows:

- LOD = 0.025 ppm
- LOQ = 0.25 ppm

Chromatographic separation between AZBT (MS chromatogram) and sartans (peaks detected in UV) is shown in Figure 5. An excellent gaussian peak was obtained for AZBT impurity on the Accucore Biphenyl column. Furthermore, all sartan API peaks were well separated from the AZBT impurity on this column; therefore, a suitable time segment was fixed for sending the LC flow to the MS from 2.4 to 5.2 minutes to detect the peak of AZBT, and the rest of the time the LC flow was diverted to the UV and subsequently to waste. The UV chromatogram overlay plot shown in Figure 5 represents the chromatography of each sartan in the corresponding tablet samples.

Table 8. Linearity plot results

Injection name	Calibration level	Nominal conc. (ng/mL)	Calculated conc. (ng/mL)	Deviation (%)	Area (counts * s)	S/N
Calibration_STD_1	1	0.25	0.24	-2.48	20793	244.4
Calibration_STD_2	2	0.50	0.52	4.83	51230	582.5
Calibration_STD_3	3	1.25	1.26	0.82	131160	1468.7
Calibration_STD_4	4	5.0	4.9	-1.11	531159	6028.2
Calibration_STD_5	5	10.0	9.9	-1.24	1066595	11920.8
Calibration_STD_6	6	20.0	19.8	-1.04	2143180	23957.6
Calibration_STD_7	7	40.0	40.1	0.22	4346996	46454.5

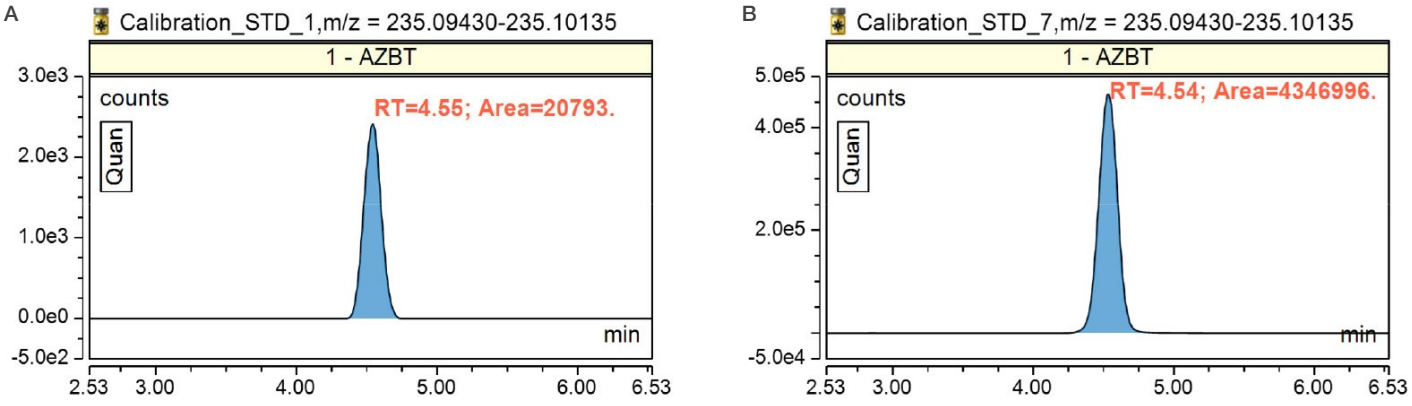

Figure 4. Chromatograms of (A) standard 1 (0.25 ng/mL) and (B) standard 7 (40 ng/mL)

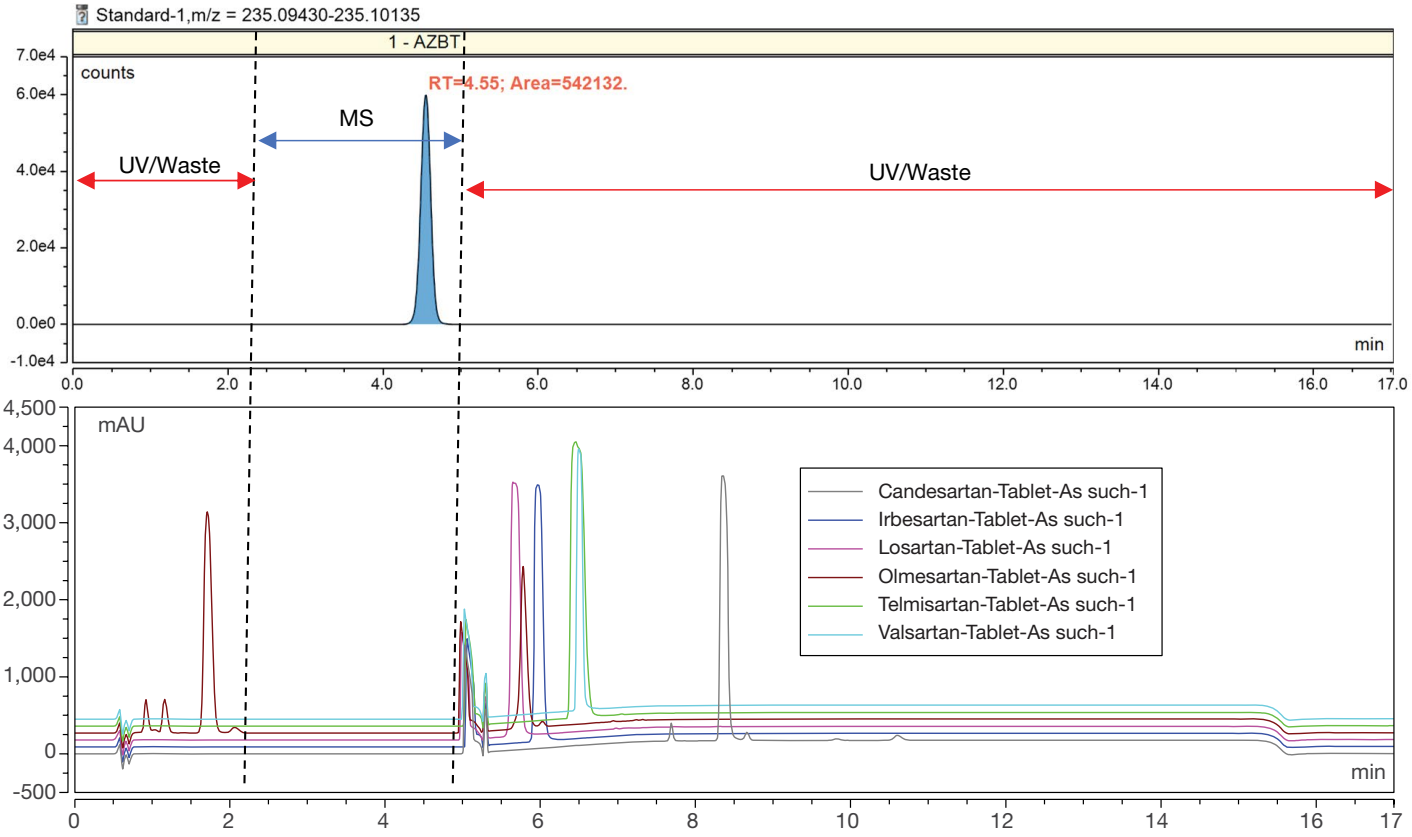

Figure 5. Chromatographic separation of AZBT and sartans

Recovery results

Recovery experiments were performed at three levels, i.e., low-level (0.5 ng/mL), mid-level (standard limit level, 5 ng/mL), and high-level concentration (40 ng/mL) for all sartan drug products. All analyses were performed in triplicate. Results of recovery studies are tabulated in Table 9.

Table 9. Recovery at low, mid, and high levels

Recovery level	Observed recoveries
Low (0.5 ng/mL)	86 to 124%
Mid (5 ng/mL)	92 to 113%
High (40 ng/mL)	95 to 105%

Mass spectra of AZBT impurity on the Orbitrap Exploris 120 MS

Orbitrap technology delivers excellent mass accuracy by virtue of high resolution. Though it is suitable to perform the analysis in either t-SIM mode (for quantitation using the precursor ion) or t-MS^2 mode (for quantitation using product ions), the selection may differ for different molecules. Figures 6a and 6b show MS^1 and MS^2 spectra, respectively, in diluent blank and 5 ng standard for AZBT impurity. These show very close masses that may interfere with the mass of interest. With an HRMS instrument, these interfering masses can be excluded after utilizing the best parameters of mass tolerance. In this note, quantification of AZBT was performed on t-MS^2 using 235.09782 as the product ion.

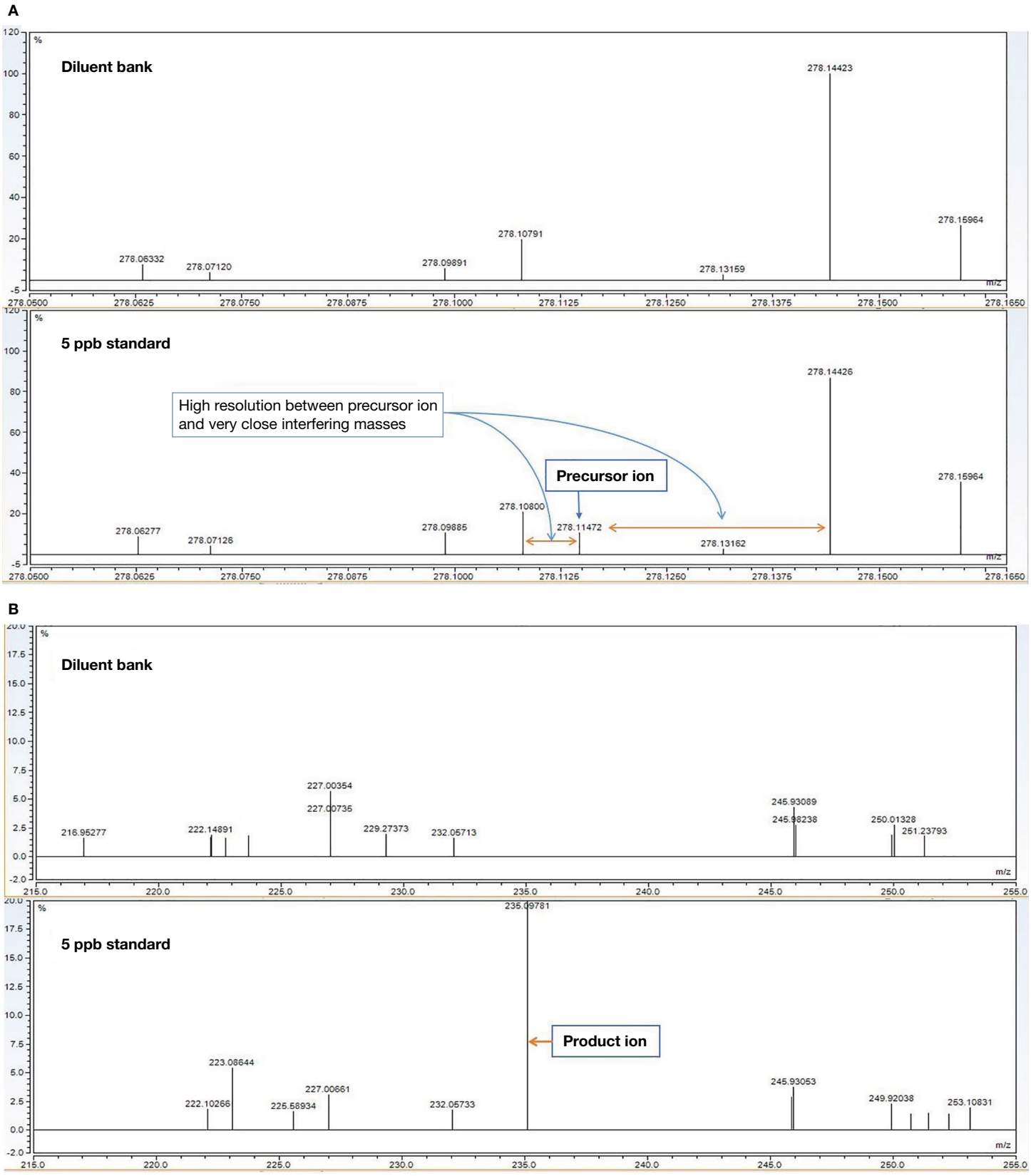

Figure 6. (A) MS¹ spectra of AZBT; (B) MS² spectra of AZBT

Conclusion

- A rapid and confident LC-HRAM-MS method for the analysis of AZBT impurity in six different sartan drug products (candesartan, irbesartan, losartan, olmesartan, telmisartan, and valsartan) has been successfully developed. The method shows capabilities to meet expectations even lower than currently required concentrations (5 ppm).
- The coefficient of correlation R^2 was found to be 0.9992.
- The LOQ for AZBT was established at par with current regulatory expectation (0.25 ppm).
- The % RSD of peak area at desired standard limit concentration (5 ppm with respect to drug substance) and at low-level concentration (0.25 ppm with respect to drug substance) was less than 0.85% and 2.7%, respectively.
- Cumulative % RSD of peak area (i.e., system suitability standard and bracketing standards) was found to be 2.5% across 128 injections (36 hours), which indicates the robustness of the system and the optimized method.
- % Recovery was determined at 5 ppm standard level and found to be within the permissible limit (70 to 130%). Recovery was determined at two additional concentration levels, i.e., 0.5 ppm level and highest concentration level of the method, and the results were found to be within 70–130% of the expected concentration.

In summary, the LC-HRAM MS system configuration containing a Vanquish UHPLC coupled with an Orbitrap Exploris 120 high-resolution, accurate mass spectrometer provides confident quantitation of AZBT impurity with excellent chromatographic separation from the sartan API peaks by utilizing a Thermo Scientific Accucore Biphenyl, 100 × 2.1 mm, 2.6 µm column. The method demonstrates excellent linearity, reproducibility, and recovery results. Therefore, with overall data consistency, this method has been found to be suitable for the analysis of AZBT in six sartans.

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