

AZBT impurity in Valsartan, Irbesartan, Losartan, Candesartan

LC-MS/MS Method (Shimadzu HPLC + AB Sciex QTrap 5500):

HPLC Parameters

Column: InfinityLab Poroshell 120 PFP, 3.0 x 100 mm, 2.7 µm, e. g. Part No: 695975-308, Agilent

Eluent A: Water LCMS-Grade + 0.1 % Formic acid (LC-MS Grade)

Eluent B: Acetonitril LCMS-Grade/Water LCMS-Grade 95/5 (V/V) + 0.1 % Formic acid (LC-MS Grade)

Oven temperature: 40 °C

Autosampler temperature: 15 °C

Flow: 0.40 ml/min

Rinsing Solvent: Methanol

Injection volume: 5 µl

Gradient:

Time	A conc.	B conc.	Right Valve Position (0: waste; 1: MS)
0.10	60	40	
3.80			1
5.50	60	40	
6.00	0	100	
6.50			0
11.00	0	100	
11.01	60	40	
12.00	60	40	

additional equilibration time: 5.00 min

MS Parameters:

Scan Type: MRM (MRM)
Polarity: Positive
Ion Source: Turbo Spray
Resolution Q1: Unit
Resolution Q3: Unit
Intensity Thres.: 0.00 cps
Settling Time: 0.0000 msec
MR Pause: 5.0000 msec

Q1 Mass (Da)	Q3 Mass (Da)	Time (msec)	ID	DP (Volts)	CE (Volts)	CXP (Volts)
277.986	234.9	100	AZBT 278/235	61	11	12
277.986	207.0	100	AZBT 278/207	61	19	12
277.986	180.0	100	AZBT 278/180	61	31	16
277.986	150.9	100	AZBT 278/151	61	57	10
282.023	239.083	100	AZBT-d4 282/239	51	11	12
282.023	211.026	100	AZBT-d4 282/239	51	19	12

Parameter Table (Period 1)

CUR: 30.00
TEM: 500.00
GS1: 40.00
GS2: 50
CAD: 9.00
IS: 5500
EP: 10.00

Reference substances:

AZBT (5-[4'-(Azidomethyl)-[1,1'-biphenyl]-2-yl]-2H-tetrazole), e. g. Biozol/TRC, Catalogue Number: N529000,
AZBT-d4 (5-[4'-(Azidomethyl)-[1,1'-biphenyl]-2-yl-2',3',5',6'-d4]-2H-tetrazole), e. g. TLC/Labmix24 No. I-0226

Stock solutions:

AZBT: approx. 10 mg/100 ml Methanol (c = approx. 100 µg/ml)

AZBT-d4: approx. 5 mg/50 ml Methanol (c = approx. 100 µg/ml)

Calibration and spiking solution:

50 µl AZBT stock solution / 20 ml of acetonitrile/water 80/20 (AZBT: c = 250 ng/ml)

ISTD solutions:

ISTD 1: 50 µl of AZBT-d4 stock solution/ 5 ml acetonitrile/water 80/20 (AZBT-d4: c = 1000 ng/ml)

ISTD 2: 1000 µl of ISTD 1 solution/ 10 ml acetonitrile/water 80/20 (AZBT-d4: c = 100 ng/ml)

Linearity (Calibration working solutions)

Description	Calibration-solution [µl]	ISTD 2-solution [µl]	Acetonitrile/Water [µl]	C _{AZBT} [ng/ml]	eq. API [ppm]
Blank + ISTD	0	100	9900	0	0
K1	10	100	9890	0,25	0,8
K2	25	100	9875	0,625	2
K3	50	100	9850	1,25	4
K4	100	100	9800	2,5	8
K5	500	100	9400	12,5	42
K6	1000	100	8900	25,0	83

Concentration of internal standard: approx. 1 ng/ml

Reference sample amount: 30 mg pure API

⇒ **result:** R^2 (AZBT) = 0,9982

1. LOQ/LOD

Limit of quantitation/Limit of detection (LOQ/LOD) AZBT -278/235:

⇒ LOQ = 0,4 ppm

⇒ LOD = 0,1 ppm

2. Sample preparation

Sample solution (API), each prepared in duplicate:

- approx. 30 mg of a homogenized sample are weighed into a plastic centrifuge tube
- addition of 100 µl of ISTD 1 solution
- addition of 9.9 ml of acetonitrile/water 80/20
- short shaking, followed by treatment for 10 minutes in an ultrasonic bath (check visually if completely dissolved)
- for quality control: a third sample was prepared in the same manner, but after the addition of 100 µl ISTD 1, 500 µl of spiking solution was added ($\pm 4 - 4.5$ ppm) + 9.4 ml of acetonitrile/water 80/20
- after filtration (RC 0,45 µm) the samples were diluted 1:10 (100 µl sample + 900 µl acetonitrile/water 80/20)

3. Specificity (to be checked for possible interferences of the API or related impurities with AZBT)

Specificity check solution:

A preferably non contaminated API sample was prepared according to 2.

⇒ No interference with AZBT signal (~5 min)

Remarks:

The method is only validated for Valsartan, Irbesartan, Losartan and Candesartan API on an AB Sciex Qtrap 5500 instrument. For other instruments or instrument parameters, it should be validated accordingly.
