Genotoxic substances in sartans

1 Objective and scope

This document describes an LC-MS/MS method for the measurement of azidomethyl biphenyl tetrazole (AZBT) and azidomethyl biphenyl carbonitrile (AZBC) in sartans.

Please note that AZBC should be quantified “for information only. For the moment, there is no regulatory obligation to determine it.
In addition, this document is the English translation of the method. The German version, available on the Swissmedic website, is the official version.

2 Principle of the method

The homogenised samples are dissolved in an 80:20 solution of acetonitrile:water. After dissolution, the samples are sonicated for 10 minutes and then centrifuged. An aliquot of the supernatant is then analysed by LC-MS/MS.

3 Validation data

See validation report ***31_VA_185 Genotoxic substances in sartans VA***

4 Context

- Not applicable

5 Definitions and abbreviations

See ***OMCL Glossary***

Term / Abbreviation	Explanation	Additional information
MRM	Multiple reaction monitoring	Selective and sensitive MS measurement mode
API	Active pharmaceutical ingredient	Active ingredient
AZBT	Azidomethyl biphenyl tetrazole	Gentoxic substance; S-3996
ISTD	Internal standard	
AZBC	Azidomethyl biphenyl carbonitrile	Genotoxic substance; S-4019
VF	Volumetric flask	

6 Documents

For specifications, see ***BPM / Processes / Tools*** or ***LIMS / Methods***

- ***31_VA_185 Genotoxic substances in sartans VA***

7 Special measures / Safety instructions

Both AZBT and AZBC have carcinogenic potential. Appropriate protective measures must be taken.

Not subject to document control
(OMCL Swissmedic)
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8 Reference and control substances, equipment, materials, reagents and solutions

8.1 Reference substances

Name	Content / Purity	S-Nr. LIMS	Manuf./Supplier / Art. No. (or equivalent)
Azidomethyl biphenyl tetrazole	98.7 %	S-3996	Toronto Research Chemicals
Azidomethyl biphenyl carbonitrile	99.1 %	S-4019	Chinoin Pharmaceutical and Chemical Works
Azidomethyl biphenyl tetrazole – d4 (Na salt)	96.1 %	S-4130	TLC Pharmaceutical Standards Ltd.
AZBT-Stock solution 31_PV_179	n/a	S-4048	n/a

8.2 Control substances

Not applicable

8.3 Equipment and materials

Name	LIMS protocol logging E: Logging for results S: Logging for substances
LC-MS/MS (e.g. 0524A)	E

8.4 Reagents

Name	LIMS No.	Manuf./Supplier / Art. No. (or equivalent)
Acetonitrile	S-2058	AppliChem GmbH / Axon Lab AG / 221881.1611
Methanol	S-1712	AppliChem GmbH / Axon Lab AG / 221091.1612
Formic acid	S-2031	Merck / Merck / 253
Milli-Q water	S-2206	OMCL

8.5 Solutions

Solution name	Preparation
AZBT stock – 1	Transfer approx. 5 mg AZBT to a 20 mL vol. flask; dilute to vol. with methanol
AZBT stock – 2	Transfer 1 mL AZBT Stock – 1 to a 10 mL vol. flask; dilute to vol. with methanol
AZBT stock – 3	Transfer 1 mL AZBT Stock – 2 to a 10 mL vol. flask; dilute to vol. with methanol
AZBT stock – 4	Transfer 1 mL AZBT Stock – 3 to a 10 mL vol. flask; dilute to vol. with methanol
AZBC stock – 1	Transfer approx. 5 mg AZBC to a 20 mL vol. flask; dilute to vol. with methanol
AZBC stock – 2	Transfer 1 mL AZBC Stock – 1 to a 10 mL vol. flask; dilute to vol. with methanol
AZBC stock – 3	Transfer 1 mL AZBC Stock – 2 to a 10 mL vol. flask; dilute to vol. with methanol
AZBC stock – 4	Transfer 1 mL AZBC Stock – 3 to a 10 mL vol. flask; dilute to vol. with methanol
AZBT-d4 ISTD stock solution	Transfer approx. 5 mg of AZBC to a 50 mL vol. flask; dilute to vol. with methanol
Diluent 1	1000 mL acetonitrile:water 80:20 + 0.5 mL AZBT-d4 ISTD stock solution
Diluent 2	Acetonitrile:water 80:20

Prepare the AZBT stock – 3 and AZBC stock – 3 solutions in duplicate in order to rule out weighing or dilution errors. Prior to processing, spike 3 blank solutions with 500 µL of each stock – 3 solution (5 ppm). The mean response measured for each spiked blank solution must not deviate by more than 5%. The solutions may be used for the analysis only if they meet this criterion.

9 Procedure

Prepare a mixed sample by grinding approximately 5 to 10 tablets. Weigh out a quantity corresponding to 250 mg of active ingredient. The maximum quantity of medicinal product to be used is 1500 mg. If necessary, the initial weight (250 mg) may be reduced without affecting the validity of the analytical procedure (minimum 30 mg).

Take up the sample with 10 mL of diluent 1, vortex briefly and then sonicate for 10 min in an ultrasonic bath. Centrifuge the sample for 10 min at 10000 *g* and a temperature of 20 °C. Transfer 100 µL of the supernatant to a vial. Add 900 µL of diluent 2 and analyse by LC-MS/MS using an injection volume of 5 µL.

Generate an external calibration curve including at least 3 points. Where possible, the curve should contain calibration points with 10%, 100% and 200% of the corresponding limit value. The calibration points may be adjusted if necessary. The lowest calibration point must not be below the corresponding LOQ.

Prepare each sample in duplicate. Spike one of these preparations with 50% of the limit value of the corresponding sartan.

If requested by the market surveillance for example, the analysis can be performed in triplicate for samples with a content of ≥ 80% of the authorised limit. A fourth analysis may also be carried out to determine the recovery in a sample spiked with approximately 50% of the value obtained in the first analysis. If the samples show higher concentrations, the calibration curve can be extended accordingly or the sample weight can be reduced.

Inject a blank after each sample. After 20 measurements and at the end of the injection sequence, verify the calibration by injecting the standard with the target concentration. Inject a blank before and after this standard.

If unexpected peaks interfere with the analysis of a sample, the test procedure can be modified in order to enhance the chromatographic separation or define a more appropriate extraction procedure to exclude interfering substances from the analysis. In this case, the modified analytical procedure must be validated in situ.

LC system

Column	InfinityLab Poroshell 120 PFP, 3.0 x 100 mm, 2.7 µm		
Mobile Phase A	Milli-Q water with 0.1% formic acid		
Mobile Phase B	Acetonitrile:Milli-Q water 95:5 (*V/V*) with 0.1% formic acid		
Autosampler temperature	15 °C		
Solution temperature	40 °C		
Injection volume	5 µL		
Flow rate	0.4 mL/min		
Gradient	RT/ min	%A	%B
	0	60	40
	0.1	60	40

	5.5	60	40
	12	0	100
	14	0	100
	14.01	60	40
	18.00	60	40
UV Detection (optional)	220 nm, 4 nm bandwidth		

MS settings

Source	ESI / positive
Scan type	MRM
MRM detection window	Unscheduled
Curtain gas (CUR)	30
Collision gas (CAD)	Medium
Temperature (TEM)	500
Ion source gas 1 (GS1)	40
Ion source gas 2 (GS2)	50
Ion spray voltage [IS]	5500
Declustering potential (DP)	61
Entrance potential (EP)	10
Collision cell exit potential (CXP)	12

MRM transitions

ID	Q1	Q3	CE	Dwell Time [msec]
AZBT – 1	277.986	234.90	11	50
AZBT – 2	277.986	207.0	19	50
AZBT-d4 – 1	282.023	239.083	11	25
AZBT-d4 – 2	282.023	211.023	19	25
AZBC – 1	207.000	151.000	46	50
AZBC – 2	192.000	165.000	30	50

Diverter valve

RT	Diverter Valve
0.1	Waste
4.4	MS
5.8	Waste
8.4	MS
9.6	Waste

10 Evaluation and measurement uncertainty

10.1 Evaluation

For AZBT, the ratios of the peak areas of the standard solutions to those of the internal standard are plotted against the concentration of the standard solutions. For AZBC, the peak areas of the standard solutions are plotted against the concentration of the standard solutions. A linear regression is created for this purpose. The linear regression is calculated with a 1/x weighting to compensate for the variance inhomogeneity over the calibrated range. This is used to calculate the concentration of the test sample. The recovery is calculated from the spiked sample. The calculated recovery in the spiked sample must be between 70% and 130%.

$$\%\ \text{recovery} = \frac{C_{sp} - C_0}{C_s} * 100$$

C_{sp}: concentration of the spiked sample [ng/mL]

C_0: concentration of the unspiked sample [ng/mL]

C_s: theoretical spiked concentration [ng/mL]

If the content is found to be above 80% of the limit, these results can be confirmed by an additional analysis in triplicate. In such cases, the mean value obtained for this triplicate analysis is shown as the result.

10.2 Measurement uncertainty

Measurement uncertainty was determined during validation and was found to be 20%.

11 Data recording

The requirements for data recording are given in the corresponding work instructions on this topic. Below are PV-specific data recording descriptions, if required.

Values below 10% of the limit value or below the LOQ are shown in the LIMS as "< (corresponding value)". Values greater than 200% of the limit are shown in the LIMS as "> (corresponding value)".

12 Quality control

- No significant interfering peaks present in the blanks
- No significant interfering co-eluting peaks present in the spiked samples
- Calibration curve correlation coefficient must be r > 0.995
- Recovery for “check-standards” and spiked samples is 70% – 130% of the theoretical value

13 Document history

Version No.:	Modification date/approval:	Change from previous version:
01	24.06.2021/meu	Creation