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Application News

Liquid Chromatograph Mass Spectrometer LCMS-8045

Quantification of Four Azido Impurities in Losartan Potassium API using LCMS-8045

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User Benefits

- A simple and robust LCMS method for the determination of four Azido impurities in Losartan Potassium API.
- No complex sample pre-treatment is required.

■ Introduction

Losartan belongs to a class of drugs called angiotensin receptor blockers (ARBs). It works by relaxing blood vessels so that blood can flow more easily. Losartan has antihypertensive and vasodilatory effects and is prescribed for the treatment of high blood pressure and to help protect the kidneys from damage due to diabetes. It is also used to lower the risk of strokes in patients with high blood pressure and an enlarged heart.

The azido impurities are derived from sodium azide, which is a precursor in the synthesis of losartan and classified as a first-class poison. The azido impurities are considered as mutagens. A mutagen is a chemical substance that can cause a change in the DNA of a cell. These mutations may increase the risk of cancer, however the specific risk for these azido impurities to cause cancer in humans is unknown.

The risk caused by these azido impurities at the levels detected in sartan medicines till date is very low. However, such contamination is considered unacceptable for a medicine. The actual health risks from these impurities depend on the dose of the medicine and will vary from person to person. Therefore, it becomes necessary to develop a highly sensitive and robust analytical method to detect azido impurities in the losartan drug substances. Considering the risk of cancer and challenges such as the structural similarities between these impurities and losartan drug substance, it is imperative to have a sensitive, reliable & accurate method for determination of azido impurities in losartan drug.

This application note describes an LC-MS/MS method for the direct quantification of azido impurities in losartan potassium API.

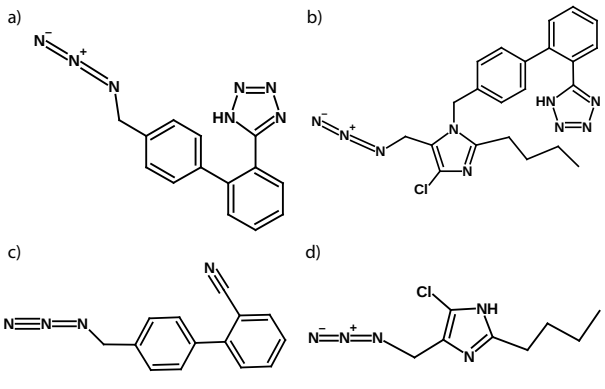

Fig. 1 Structure of a) AZBT; b) AZLS; c) AZBC; d) and AZIM

■ Experimental

Four azido impurities namely, 5-[4'-(Azidomethyl)[1,1'-biphenyl]-2-yl]-2H-tetrazole (AZBT), 5-(4'-((5-(Azidomethyl)-2-butyl4-chloro-1H-imidazol-1-yl)methyl)-[1,1'-biphenyl]-2-yl)-1H-tetrazole (AZLS), 4'-(Azidomethyl)-[1,1'-biphenyl]- 2-carbonitrile (AZBC) and 5-(Azidomethyl)-2-butyl-4- chloro-1H-imidazole (AZIM); as shown in Fig. 1, were analyzed to perform steps such as precursor ion selection and MRM optimization. An LC-MS/MS method with optimum MRMs and their CEs was generated in segments with Ultra High-Performance Liquid Chromatography (UHPLC) Nexera™ XS coupled with LCMS-8045, a triple quadrupole mass spectrometer from Shimadzu Corporation, Japan (Fig. 2). Table 1 shows the optimized MRM transitions used for further analysis.

LCMS-8045, 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.

Fig. 2 Nexera™ XS with LCMS-8045 system

■ Linearity of the azido impurities

Five-point calibration curves for all azido impurities were prepared in diluent (water : methanol 10:90 v/v) and analyzed using the analytical conditions described in Table 2. A divert valve was employed to direct the losartan peak into waste and to protect LCMS from the contamination. A representative UV chromatogram of losartan potassium API and its overlapping LCMS chromatogram of azido impurities is shown in Fig. 3.

Table 1 MRM transitions of azido impurities

Azido Impurity	MRM
AZBT	278>235
AZLS	448>405
AZBC	252>207
AZIM	214>171

Table 2 Analytical Conditions

HPLC system	:	Nexera™ XS
Column	:	Shim-pack™ Scepter Phenyl-120 (150 mm x 4.6 mm, 3µ) (P/N :227-31067-05)
Column oven	:	45 °C
Mobile phases	:	A - 10 mM Ammonium formate in water B - Methanol
Flow rate	:	0.4 mL/min
Gradient program (%B concentration)	:	0-8 min 60 %B; 8-9 min 60 to 95 %B; 9-17 min 95 %B; 17-17.1 min 95 back to initial 60 %B; 18 min STOP.
Injection volume	:	5 µL
Detector	:	LCMS-8045
Interface	:	ESI
Interface voltage	:	4.5 kV
Temperature	:	Interface: 350 °C Desolvation Line: 250 °C Heater Block: 300 °C
Gas flow	:	Nebulizing Gas: 3 L/min Drying Gas: 5 L/min Heating Gas: 10 L/min

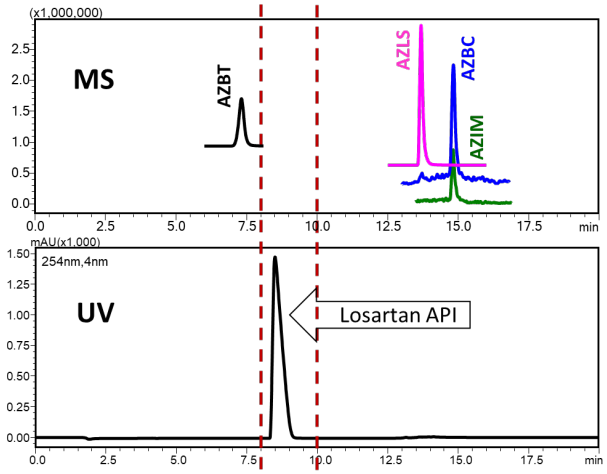

Fig. 3 Representative UV chromatogram of Losartan drug substances and its overlapping with MRM chromatogram of azido impurities

Fig. 4, 5, 6, and 7 depict the calibration curve, overlay of linearity standards & LOQ solution chromatograms for AZBT, AZLS, AZBC, and AZIM impurities, respectively. The range of calibration curves for azido impurities was from 1 to 16.0 ppb i.e. 0.667 ppm to 10.667 ppm with reference to sample concentration of 1.5 mg/mL.

AZBT

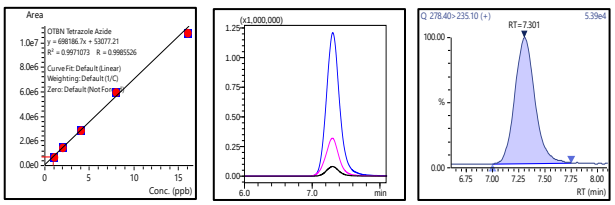

Fig.4 Calibration curve, overlay of linearity standards & LOQ solution chromatogram for AZBT

AZLS

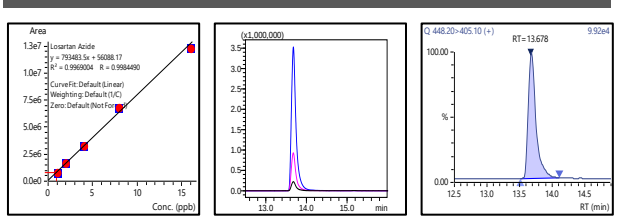

Fig.5 Calibration curve, overlay of linearity standards & LOQ solution chromatogram for AZLS

AZBC

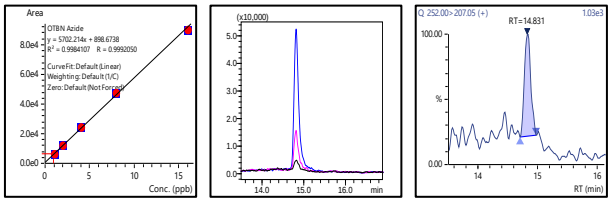

Fig.6 Calibration curve, overlay of linearity standards & LOQ solution chromatogram for AZBC

AZIM

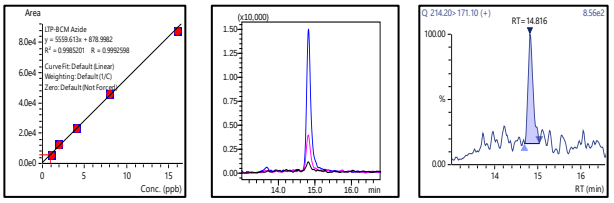

Fig.7 Calibration curve, overlay of linearity standards & LOQ solution chromatogram for AZIM

The LOQ for all azido impurities was found to be 0.667 ppm with reference to sample concentration of 1.5 mg/mL, based on S/N. The S/N ratio, coefficient of regression and % RSD at LOQ are shown in Table 3.

Table 3 Summary of linearity & LOQ results

Azido Impurity	R^2	LOQ		
		Conc.* (ppm)	%RSD (n=6)	S/N
AZBT	0.997	0.667	0.50	1098
AZLS	0.996	0.667	0.79	516
AZBC	0.998	0.667	9.29	10
AZIM	0.998	0.667	5.01	10

* Concentration with reference to sample at 1.5 mg/mL.

■ Sample Analysis

1. Weigh 7.5 mg of losartan potassium API in two different 5 mL standard volumetric flask.
2. Add 2.5 mL of diluent to one flask (sample blank) and 2.5 mL diluent spiked with azido impurity standards to the second flask (recovery sample).
3. Sonicate the samples till they dissolve and make up the volume to 5 mL.
4. Filter the solution using a 0.45 µm nylon syringe filter and analyze the samples using LC-MS/MS.

■ Results

The results of sample analysis are shown in Table 4. The content of AZLS in losartan potassium API was found to be more than 400 ppm, hence AZLS impurity was not considered for recovery study. The results of recovery analysis are shown in Table 5.

Table 4 Summary of Sample Analysis

Azido Impurity	Amount found in the API (ppm)*
AZBT	3.55
AZLS	409.69
AZBC	BLOQ
AZIM	BLOQ

* Concentration with reference to sample at 1.5 mg/mL.

Table 5 %Recovery at LOQ

Azido Impurity	Amt. of Impurity in Sample (ppm)	Amt. of Impurity Spiked (ppm)	Amt. of Impurity in Spiked Sample (ppm)	% Recovery
AZBT	3.55	0.667	4.32	115
AZBC	BLOQ	0.667	0.85	127
AZIM	BLOQ	0.667	0.54	81

■ Conclusion

- A LC-MS/MS quantification method for the four azido impurities in Losartan drug substance has been successfully developed on the Shimadzu LCMS-8045 system.
- Five levels of linearity was performed for all the impurities and regression coefficient was greater than 0.99.
- The repeatability (n=6) at LOQ level was found to be less than 10 %RSD.
- Losartan potassium API was found to contain 3.55 ppm of AZBT and 409.69 ppm of AZLS.
- Recovery analysis was performed, and it matched to the acceptance criteria between 70 to 130 %.

BLOQ = Below limit of quantitation

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