LOSARTAN Potassium

LC-MS/MS Method ´190321_NMBA_APCI_POS_waste´ (Shimadzu HPLC + Sciex QTrap 5500):

HPLC Parameters

Column: Luna® 5 µm C8(2) 100 Å, LC Column 150 x 2 mm, Part No: 00F-4249-B0, Phenomenex

Security Guard Cartridges: C8 4 x 2.0 mm ID, Part No: AJ0-4289, Phenomenex

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

Eluent B: Methanol LCMS grade + 0.1 % Formic acid (LC-MS Grade)

Oven temperature: 40 °C

Autosampler temperature: 15 °C

Flow: 0.40 ml/min

Rinsing Solvent: Methanol / Water 65/35 (V/V)

Injection volume: 15 µl

Gradient:

Time	A conc.	B conc.	Right Valve Position (0: waste; 1: MS)
0.00	80	20	
1.00			1
3.00	80	20	
4.50			0
6.00	5	95	
11.00	5	95	
12.00	80	20	

additional equilibration time: 5.00 min

MS Parameters:

Scan Type: MRM (MRM)
Polarity: Positive
Ion Source: Heated Nebulizer
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)
147.008	**117.0**	**250.0**	**NMBA_147/117**	**46**	**9**	**8**
147.008	44.0	250.0	NMBA_147/44	46	17	22
147.008	86.9	250.0	NMBA_147/87	46	15	8
149.97	120.1	250.0	NMBA-d3_150/120	36	9	8
149.97	87.0	250.0	NMBA-d3_150/87	36	17	10
149.97	**47.1**	**250.0**	**NMBA-d3_150/47**	**36**	**15**	**10**

Parameter Table(Period 1)
CUR: 30.00
TEM: 350.00
GS1: 35.00
CAD: 6.00
NC: 2.00
EP 10.00

Reference substances:

NMBA (N-Nitroso-N-methyl-4-aminobutyric acid), Biozol/TRC, Catalogue Number: N529000,
NMBA-d3 (N-Nitroso-N-methyl-4-aminobutyric acid), BOC Sciences

Stock solutions:

NMBA: approx. 10 mg/ 10 ml Water (c = approx. 1000 µg/ml)
NMBA-d3: approx. 2,5 mg/ 2 ml Water (c = approx. 1250 µg/ml)

Stock solutions were prepared in portions and stored at - 20 °C. Ongoing stability studies show the stability of the solutions under these conditions for at least one week (8 d).

Calibration and spiking solution:

25 µl NMBA stock solution / 50 ml of water (NMBA: c = 500 ng/ml)

ISTD solution:

From ISTD stock solutions: 20 µl of NMBA-d3 stock solution/ 50 ml Water (NMBA-d3: c = 500 ng/ml)

Linearity (Calibration working solutions)

Description	Calibration-solution [µl]	ISTD- solution [µl]	Water [µl]	C_{NMBA} [ng/ml]	eq. 50 mg API [ppb]
Blank + ISTD	0	100	9900	0	0
K1	4	100	9896	0,2	40
K2	10	100	9890	0,5	100
K3	20	100	9880	1	200
K4	40	100	9860	2	400
K5	200	100	9700	10	2,000
K6	400	100	9500	20	4,000

Concentration of internal standard: approx. 5 ng/ml

Reference sample amount: 50 mg pure API (Losartan-K)

⇒ **result: R^2 (NMBA) = 1,0**

1. LOQ/LOD

Limit of quantitation/Limit of detection (LOQ/LOD) NMBA -147/117:

S/N (1,5024 ng/ml) = 105

⇒ LOQ = 28,6 ppb
⇒ LOD = 8,6 ppb

2. Sample preparation

Sample solution (real samples), each prepared in duplicate:

- approx. 50 mg of a homogenized sample are weighed into a plastic centrifuge tube
- addition of 100 µl of ISTD solution
- addition of 9.9 ml of ultrapure water
- vortexing, followed by treatment for 15 minutes in an ultrasonic bath (check visually if completely dissolved)
- membrane filtration into a HPLC vial
- for quality control: a third sample was prepared in the same manner, but after the addition of ISTD, 60 µl of spiking solution was added (+ 9.840 ml of ultrapure water)

3. Specificity

Specificity check solution:

A non contaminated Losartan API sample matrix was prepared according to 2.

⇒ No interference with NMBA signal (~2 min)

4. Precision/Accuracy

Sample solution (spiked samples):

- approx. 50 mg of a homogenized sample of the finished product are weighed into a plastic centrifuge tube
- addition of 100 µl of ISTD solution
- addition of 30 µl spiking solution (3 times), or 60 µl spiking solution (6 times), or 90 µl spiking solution (3 times)
- addition of 9.870 or 9.840, or 9.810 ml of ultrapure water (depends on volume of spiking solution
- vortexing, followed by treatment for 15 minutes in an ultrasonic bath (check visually if completely dissolved)
- membrane filtration into a HPLC vial

Remarks:

The method is only validated for Losartan-K API.