25.10.2022

N-Nitroso-Ramipril in Ramipril tablets

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

HPLC Parameters

Column: XTerra MS C_{18} 3.5 µm, 3.0 x 100 mm, Waters; Part.-No.: 186000418

Security Guard Cartridges: C_{18} 4 x 2.0 mm, Part No: AJO-2486, Phenomenex

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

Eluent B: Acetonitril LCMS grade / H_2O 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: Acetonitril / Water 80/20 (V/V)

Injection volume: 5 µl

Gradient:

Time	A conc.	B conc.	Right Valve Position (0: waste; 1: MS)
0.00	60	40	
3.00	60	40	
4,80			1
10	0	100	
10.1			0
13.5	0	100	
14.5	60	40	
15.0	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)
446.15	**234.1**	**250**	**Nitrosoramipril_446/234**	**81**	**27**	**18**
446.15	156.1	250	Nitrosoramipril_446/156	81	17	12
468.10	**234.1**	**100**	**Nitrosoquinapril_468/234**	**81**	**25**	**12**
468.10	178.1	100	Nitrosoquinapril_468/178	81	15	16

Parameter Table(Period 1)
CUR: 30.00
TEM: 500.00
GS1: 45.00
GS2 50.00
CAD: 6.00
IS: 5500
EP 10.00

Reference substances:

N-Nitroso-Ramipril, Art.-Nr.: R-10429-01, # NITR-1491-5102-36A, Acanthus research
N-Nitroso-Quinapril, Art.-Nr.: Q-0215, # 4925-032A3, TLC

Stock solutions:

Nitrosoramipril: approx. 10 mg/100 ml methanol (c = approx. 100 µg/ml)
Nitrosoquinapril: approx. 5 mg/50 ml methanol (c = approx. 100 µg/ml)

Stock solutions were stored at 2-8 °C. Ongoing stability studies show the stability of the solutions under these conditions for at least two months.

Calibration and spiking solution:

50 µl Nitrosoramipril stock solution/25 ml of acetonitrile/water 80/20 (c = 200 ng/ml)

ISTD solution:

50 µl Nitrosoquinapril stock solution/25 ml acetonitrile/water 80/20 (c = 200 ng/ml)

Linearity (Calibration working solutions)

Description	Calibration-solution [µl]	ISTD- solution [µl]	ACN/Water 80/20 [µl]	$C_{Nitrosoramipril}$ [ng/ml]
Blank + ISTD	0	100	9900	0
K1	4	100	9896	0,08
K2	10	100	9890	0,2
K3	25	100	9875	0,5
K4	50	100	9850	1,0
K5	200	100	9700	4,0
K6	400	100	9500	8,0

Concentration of internal standard: approx. 2 ng/ml

Reference sample: Ramipril 10 mg Tabletten

1. LOQ/LOD

Limit of quantitation/Limit of detection (LOQ/LOD) Nitrosoramipril [446/234]:

S/N (0.10213 ng/ml) = 20

⇒ LOQ = ppm/MDD
⇒ LOD = ppm/MDD

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 50 µl pyrrolidine solution (approx.10 mg/ml in methanol)
- addition of 100 µl of ISTD solution

- addition of 9.9 ml of ultrapure acetonitrile/water 80/20
- vortexing, followed by treatment for 15 minutes in an ultrasonic bath
- membrane filtration into a HPLC vial
- for quality control: a third sample was prepared in the same manner, but after the addition of ISTD, 25 µl of spiking solution was added (+ 9.825 ml of ultrapure acetonitrile/water 80/20)

3. Specificity

Specificity check solution:

A non-spiked validation sample was prepared (N-Nitrosoramipril content: 1.0 ppm/MDD)

⇒ No interferences of the analyte signal with Ramipril or the matrix of the tablet

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 50 µl pyrrolidine solution (approx.10 mg/ml)
- addition of 100 µl of ISTD solution
- addition of 10 µl spiking solution (3 times), or 20 µl spiking solution (6 times), or 30 µl spiking solution (3 times)
- addition of 9.840 or 9.830, or 9.820 ml of ultrapure acetonitrile/water 80/20 (depends on volume of spiking solution
- vortexing, followed by treatment for 15 minutes in an ultrasonic bath
- membrane filtration into a HPLC vial

Remarks:

The method is validated for the determination of N-Nitrosoramipril in Ramipril IR tablets. For other dosage forms it must be suitably validated.