N-Nitroso-Fluoxetine in Fluoxetine tablets

LC-MS/MS Method (Shimadzu HPLC + 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)
0.0	60	40	
3.0	60	40	
8,0			1
10,0	0	100	
11,0			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)
339,14	**177,0**	**250**	**Nitrosofluoxetin_339/177**	**66**	**13**	**10**
339,14	117,10	200	**Nitrosofluoxetin_339/117**	66	27	10

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-Fluoxetine, Toronto Research Chemicals, TRC-N171730, #11-MSI-20-2

Stock solution:

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

Stock solutions were stored at 2-8 °C.

Calibration and spiking solutions:

Cal.-Sol.1: 50 µl Nitrosofluoxetine stock solution/100 ml of acetonitrile/water 80/20 (c = 50 ng/ml)
Cal.-Sol.2/spiking solution: 50 µl Nitrosofluoxetine stock solution/25 ml of acetonitrile/water 80/20 (c = 200 ng/ml)

Linearity (Calibration working solutions)

Description	Cal.-Sol. 1 [µl]	Cal.-Sol. 2 [µl]	ACN/Water 80/20 [µl]	$c_{Nitrosofluoxetine}$ [ng/ml]
K1	10	-	9990	0.05
K2	20	-	9980	0.1
K3	40	-	9960	0.2
K4	-	25	9975	0.5
K5	-	50	9950	1.0
K6	-	200	9800	4.0
K7	-	400	9600	8.0

Reference sample: Fluoxetine 10 mg Tabletten

1. LOQ/LOD

Limit of quantitation/Limit of detection (LOQ/LOD) Nitrosofluoxetin [339/177]:

S/N (0.06218 ng/ml) = 12

$\Rightarrow$ LOQ = 0.09 ppm/MDD
$\Rightarrow$ LOD = 0.03 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 of pyrrolidine solution (approx. 10 mg/ml in methanol)
- addition of 9.95 ml of ultrapure acetonitrile/water 80/20
- vortexing, followed by treatment for 10 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 50 µl Pyrrolidine solution, 40 µl of Cal.-Sol.2 (= spiking solution) was added (+9,91 ml of ultrapure acetonitrile/water 80/20)

3. Specificity

Specificity check solution:

A non-spiked validation sample was prepared and MS was run in full scan mode.

⇒ No interferences of the analyte signal with Fluoxetine 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 of pyrrolidine solution (approx.10 mg/ml in methanol)
- addition of 20 µl spiking solution (3 times), or 40 µl spiking solution (6 times), or 60 µl spiking solution (3 times)
- addition of 9.93 or 9.91, or 9.89 ml of ultrapure acetonitrile/water 80/20 (depends on volume of spiking solution
- vortexing, followed by treatment for 10 minutes in an ultrasonic bath
- membrane filtration into a HPLC vial

Remarks:

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