

Determination of *N*-nitroso duloxetine in duloxetine preparations with LC-MS

1 Objective and scope

The present method is used to quantify *N*-nitroso duloxetine in various duloxetine preparations using LC-MS/MS.

2 Principle of the method

Various preparations are extracted with acetonitrile and the nitrosoduloxetine content is determined using an external calibration curve. Two preparations are made per sample, with one preparation being spiked with *N*-nitroso duloxetine.

N-nitroso duloxetine exists in only one isomer. For calibration, the peak area is plotted against the concentration.

3 Validation data

See *in situ* validation.

4 Context

- Method development A-026103

5 Definitions and abbreviations

See **OMCL glossary**

Term / Abbreviation	Description	Additional information
MRM	Multiple Reaction Monitoring	Selective and sensitive MS measuring mode

6 Additional documents

For specific instructions, see **BPM / Procedures / Tools** or **LIMS / Methods**

None.

7 Special measures / Safety instructions

N-nitroso duloxetine is potentially carcinogenic. Appropriate protective measures must be taken.

8 Reference and control substances, test equipment, materials, chemicals and solutions

8.1 Reference material

Name	Content / purity	S-No. LIMS	Manufacturer/supplier/article no. (or equivalent)
<i>N</i> -nitroso duloxetine	0.98	S-4560-001	LGC Standards/Toronto Research Chemicals / 1-MJA-55-7

8.2 Control substances

Not applicable.

8.3 Equipment and materials

Name	LIMS procedure documentation E: documentation of results S: documentation of substances used
LC-MS/MS (e.g. MS (AB Sciex QTRAP 5500) / 0524A	E

8.4 Chemicals

Name	LIMS S-No.	Manufacturer/supplier/art. no. (e.g.)
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	Procedure	Shelf life / storage temp.
<i>N</i> -nitroso duloxetine stock solution	Weigh approx. 1 mg nitrosoduloxetine into a 1.5 mL HPLC vial and dilute to 1 mL with acetonitrile	7 days at -20°C
<i>N</i> -nitroso duloxetine Dil 0	Dilute 100 µL of the stock solution to 10 mL with acetonitrile (in a volumetric flask)	2 days at 2-8°C
<i>N</i> -nitroso duloxetine Dil 1	Dilute 100 µL of Dil 0 solution to 10 mL with acetonitrile (in a volumetric flask)	2 days at 2-8°C

To rule out errors in the weighing or dilution of the solution, the stock solution and diluted solutions (Dil 0/1/2) are prepared in duplicate (A/B). As a control, a solution of the calibration curve (approx. 1.5 ppm level) from preparation series B is prepared in duplicate and compared with the calibration curve from preparation A. The calculated concentration of the sample must not deviate by more than $\pm 5\%$ from the target concentration.

9 Procedure

A mixed sample is prepared from 6 tablets. Weigh out a quantity of the mixed sample corresponding to 3 mg of active ingredient. However, the maximum quantity of medicinal product to be used is 150 mg. Two preparations are made per sample, one of which is spiked with *N*-nitroso duloxetine (approx. 3 ppm). If a measured value lies outside the calibration range, the sample mass is either increased or decreased. The mass is changed so that the expected measured value lies within the calibration curve.

For quantification, a linear calibration curve with 6 points is produced over the range from approx. 1 ng/mL to approx. 50 ng/mL (approx. 0.3 - 17 ppm). The peak area is plotted against the concentration.

To check the device performance, the reference standard (calibration solution 4) is re-analysed at regular intervals.

LC system

Column	Luna 3u Phenyl-Hexyl, 50x2 mm, 3 µm		
Mobile phase A	Milli-Q water with 0.1% formic acid		
Mobile phase B	Acetonitrile with 0.1% formic acid		
Autosampler temperature	5°C		
Column temperature	40°C		
Injection volume	5 µL		
Flow rate	0.3 mL/min		
Gradient	RT/ min	%A	%B
	0	50	50
	1.0	50	50
	4.0	35	65
	4.1	0	100
	4.6	0	100
	4.7	50	50
	7.0	50	50
UV Detection (optional)	220 nm, 4 nm bandwidth		

MS settings

Source	ESI / positive
Scan type	MRM
MRM detection window	Unscheduled
Curtain gas (CUR)	35
Collision Gas (CAD)	Medium
Temperature (TEM)	300
Ion Source Gas 1 (GS1)	40
Ion Source Gas 2 (GS2)	20
Ion Spray Voltage [IS]	5500
Entrance Potential (EP)	10
Collision Cell Exit Potential (CXP)	13
Declustering Potential (DP)	60

MRM transitions

ID	Q1	Q3	CE	Dwell time [msec]
NA_Duloxetine_Quant	183.1	123.1	14	200
NA_Duloxetine_Qual1	327.0	183.1	9	200
NA_Duloxetine_Qual2	327.0	123.1	24	200

N-nitroso duloxetine elutes at the retention time of 3.53 min.

Diverter valve

RT	Diverter valve
0.0	to waste
2.0	to MS
5.0	to waste

10 Evaluation and measurement uncertainty

10.1 Evaluation

To determine the *N*-nitroso duloxetine content, the area of the unspiked sample is converted into the concentration using the calibration line (6-point).

The following formulae are used to calculate the recovery:

$$recovery \% = \frac{C_{sp} - \frac{C_0 \cdot M_{sp}}{M_0}}{C_s} \cdot 100$$

CBS

- C_{sp} : calculated concentration of the spiked sample / ng/mL
- C_0 : calculated concentration of the unspiked sample / ng/mL
- M_{sp} : mass of the spiked sample / mg
- M_0 : mass of the unspiked sample / mg
- C_s : concentration of the added spike in the sample / ng/mL

10.2 Measurement uncertainty

Calculated as part of the *in situ* validation.

11 Documentation and protocols

The requirements for documentation are given in the corresponding operating instructions on this topic. If necessary, specific requirements for documentation of process variable parameters are listed below.

No specific documentation is required for process variables.

12 Quality control

For quality control purposes, SST criteria are defined for each test series as well as for each sample.

SST criteria for analytical test series

Parameters	Criterion
Linearity Blank spikes (start of the sequence)	$r \geq 0.995$
Recovery reference standard	70% - 130%

SST criteria for Sample

Parameters	Criterion
Spike recovery	70% - 130%
Qualifier ratios	$\pm 25\%$

13 Document history

The last 5 version changes are shown on the document; previous versions must be deleted from the table.

Version no:	Modification date/approval:	Change from previous version:
01		Creation