

LC-MS/MS Method for the determination of NDEA and NMDA in Valsartan, Irbesartan and Losartan APIs and finished dosage forms (developed on a AB Sciex QTrap 5500):

HPLC Parameters

Column: XTerra MS C18 3,5 µm, 3,0 x 100 mm (Waters)

Security Guard: C18 4 x 3,0 mm, Part No: AJO-287 (Phenomenex)

Eluent A: Water LCMS grade

Eluent B: Methanol LCMS grade

Oven temperature: 30 °C

Autosampler temperature: 15 °C

Flow: 0.40 mL/min

Gradient:

Time	A conc.	B conc.
0.00	95	5
3.00	95	5
13.00	40	60
14.00	5	95
19.00	5	95
20.00	95	5

additional equilibration time: 5.00 min

Injection volume: 15 µL

MS Parameters:

Period 1:

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: 5.0070 msec

Q1 Mass (Da)	Q3 Mass (Da)	Time (msec)	ID	DP (Volts)	CE (Volts)	CXP (Volts)
75.048	44.003	250.0	NDMA_75/43	40	19	20
75.048	44.000	250.0	NDMA_75/44	40	17	20
75.048	58.080	250.0	NDMA_75/58	40	15	8
81.078	46.037	250.0	NDMA-d6_81/46	60	21	8
81.078	64.058	250.0	NDMA-d6_81/64	60	19	8

Parameter Table(Period 1)

CUR: 20.00
TEM: 350.00
GS1: 35.00
GS2: 50.00
CAD: 6.00
NC: 3.00
EP 10.00

Period 2

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: 5.0070 msec

Q1 Mass (Da)	Q3 Mass (Da)	Time (msec)	ID	DP (Volts)	CE (Volts)	CXP (Volts)
103.047	29.037	250.0	NDEA_103/29	60	21	14
103.047	47.030	250.0	NDEA_103/47	60	19	24
103.047	75.010	250.0	NDEA_103/75	60	13	8
113.065	34.046	250.0	NDEA-d10_113/34	60	25	16
113.065	81.047	250.0	NDMA-d6_113/81	60	15	12

Parameter Table(Period 2)

CUR: 25.00
TEM: 350.00
GS1: 35.00
GS2: 50.00
CAD: 6.00
NC: 2.00
DP: 60.00
EP: 10.00

Reference substances:

NDMA (N-Nitrosodimethylamine), Dr. Ehrensdofer, C15604000, 0,1g
NDMA-d6 (N-Nitrosodimethyl-d10-amine), CDN-Isotops, D-2937, 0,1g
NDEA (N-Nitrosodiethylamine), Sigma Aldrich, 73861-10ML, 10 mL
NDEA-d10 (N-Nitrosodiethyl-d6-amine), CDN-Isotops, D-4107, 0,1g

Stock solutions:

NDEA, NDMA, NDEA-d10, NDMA-d6: approx. 10 mg/ 10 mL MeOH (c = approx. 1000 µg/mL)

Calibration and spiking solution:

250 µL NDMA stock solution + 25 µL NDEA stock solution / 50 mL of water (NDMA: c = 5000 ng/mL;
NDEA: c = 500 ng/mL)

ISTD solution:

From ISTD stock solutions: 25 µL of each stock solution/ 50 ml MeOH (c = 500 ng/mL)

Linearity (Calibration working solutions)

Description	Calibration- solution [µL]	ISTD- solution [µL]	MeOH [µL]	Water [µL]	C _{NDEA} [ng/mL]	C _{NDMA} [ng/mL]
Blank + ISTD	0	100	400	9500	0	0
K1	4	100	400	9496	0,2	2
K2	10	100	400	9490	0,5	5
K3	20	100	400	9480	1	10
K4	40	100	400	9460	2	20
K5	200	100	400	9300	10	100
K6	400	100	400	9100	20	200

Concentration of internal standard: approx. 5 ng/mL in each case

Reference sample amount:

For Losartan:

100 mg homogenized sample of the finished product (~ 25 mg Losartan-K), **or** 25 mg pure API

For Irbesartan:

100 mg homogenized sample of the finished product (~ 75 mg Irbesartan), **or** 50 mg pure API

For Valsartan:

100 mg homogenized sample of the finished product (~ 75 mg Valsartan), **or** 50 mg pure API

1. LOQ/LOD

Limit of quantitation/Limit of detection (LOQ/LOD) NDMA -75/58

Limit of quantitation/Limit of detection (LOQ/LOD) NDEA-103/75

NDMA

	Irbesartan	Valsartan	Losartan
LOQ	79 ppb	236 ppb	492 ppb
LOD	24 ppb	71 ppb	148 ppb
Limit	320 ppb	300 ppb	640 ppb

NDEA

Irbesartan	Valsartan	Losartan
19,5 ppb	61 ppb	149 ppb
6 ppb	18 ppb	45 ppb
88 ppb	82 ppb	177 ppb

2. Sample preparation

Sample solution, each prepared in duplicate:

- approx. 100 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 400 µL of MeOH
- vortexing, followed by treatment for 30 minutes in an ultrasonic bath
- addition of 9.5 mL of ultrapure water
- vortexing, followed by treatment for 30 minutes in an ultrasonic bath
- ultracentrifugation (4000 x g; 20 °C; 10 min) of the sample followed by membrane filtration (Chromafil RC-45/15 MS, Macherey-Nagel) into a HPLC vial
- for quality control: a third sample was prepared in the same manner, but after the addition of ISTD 20 µL of spiking solution was added (+ 9.480 mL of ultrapure water)