20.11.2023

NTTP in Sitagliptin / Sitagliptin/Metformin tablets

GC-MS/MS Method (Agilent GC 8890 + GC/TQ 7010B HES):

GC Parameters

Column: Agilent HP-5 MS Ultra inert (30 m x 0.25 mm ID, 0.25 µm film)

MS parameters

NTTP: Quantifier: 221 -> 191; Qualifier: 221 -> 42

NTTP-$^{15}ND_4$: Quantifier: 226 -> 195; Qualifier: 226 -> 46

(Further parameters see attachment 1)

Reference substances:

- NTTP (7-Nitroso-3-(trifluoromethyl)-5,6,7,8-tetrahydro-[1,2,4]triazolo[4,3-a]pyrazine, CAS: [2892260-32-9], e. g. TRC, Catalogue-Number: N791810
- Internal standard: NTTP-$^{15}ND_4$ (N-(^{15}N-Nitroso)-3-(trifluoromethyl)-5,6,7,8-tetrahydro-[1,2,4]triazolo[4,3-a]pyrazine-D_4), e. g. TRC, Catalogue-Number: N526911

Stock solution:

NTTP: approx. 2 mg/10 ml Methanol (c = approx. 200 µg/ml)
NTTP-$^{15}ND_4$: approx. 2 mg/10 ml Methanol (c = approx. 200 µg/ml)

Stock solutions were stored at 2-8 °C. They are stable for 4 weeks, if stored under these conditions.

Calibration and spiking solutions:

WS1: 200 µl NTTP stock solution/20 ml of Methylene chloride (c = 2,000 ng/ml)
WS2: 1 ml WS1/10 ml of Methylene chloride (c = 200 ng/ml)

ISTD working solution: 100 µl NTTP-$^{15}ND_4$-stock solution/20 ml of Methylene chloride (c = 1,000 ng/ml)

Linearity (Calibration working solutions)

Description	WS 1 [µl]	WS 2 [µl]	ISTD-WS [µl]	Methylene chloride [µl]	C_{NTTP} [ng/ml]
K1	-	25	100	9875	0.5
K2	-	50	100	9850	1
K3	-	100	100	9800	2
K4	-	250	100	9650	5
K5	100	-	100	9800	20
K6	250	-	100	9650	50
K7	500	-	100	9400	100

Reference sample: 100 mg Filmtabletten (Sitagliptin)

1. LOQ/LOD

Limit of quantitation/Limit of detection (LOQ/LOD) NTTP [221/191]:

⇒ LOQ = 0.04 ppm/MDD*

⇒ LOD = 0.01 ppm/MDD*

*: MDD = max. daily dose ≙ 100 mg API; AI: 37 ng/day ≙ 0.37 ppm/MDD

2. Sample preparation

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

- approx. equivalent 25 mg API of a homogenized sample (here: 100 mg) are weighed into a centrifuge tube
- addition of 50 µl of ISTD working solution
- addition of 5.0 ml Methylene chloride
- shake for 30 minutes, afterwards treat for further 30 min with the help of ultrasonic, centrifuge at 4,500 rpm for 5 min, transfer the clear supernatant into a GC vial; filter with RC filter, if necessary
- for quality control: a third sample is prepared in the same manner, but before the addition of ISTD, 20 µl of WS1 are added

3. Specificity

Specificity check solution:

A non-spiked validation sample was prepared (NTTP content: c ~ 0.15 ppm/MDD). Run the MS in full scan mode (~ m/z 40 - 400)

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

4. Precision/Accuracy

Sample solution (spiked samples):

- approx. equivalent 25 mg API of a homogenized sample (here: 100 mg) are weighed into a centrifuge tube
- addition of 50 µl of ISTD working solution
- addition of 10 µl WS1 (3 times), or 20 µl of WS1 (6 times), or 30 µl of WS1 (3 times)
- addition of 5.0 ml Methylene chloride
- shake for 30 minutes, afterwards treat for further 30 min with the help of ultrasonic, centrifuge at 4,500 rpm for 5 min, transfer the clear supernatant into a GC vial

Remarks:

The method is validated for the determination of NTTP in Sitagliptin IR and Sitagliptin/Metformin IR tablets. For other dosage forms, it must be suitably validated.