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THE DETERMINATION OF N-NITROSO SILODOSIN IMPURITY (NNSI) IN SILODOSIN USING A SENSITIVE LC-MS/MS METHOD

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ABSTRACT

The current method explains the ppm level quantification of N-Nitroso silodosin in Silodosin drug substance by LC-MSMS. Using the Sciex-4500 Q-trap LC-MSMS instrument, a sensitive methodology was settled to detect NNSI (N-nitroso silodosin) impurity in silodosin drug material with appropriate precision, LOD (Limit of Detection), accuracy, solution stability, and LOQ (Limit of Quantification). In the YMC Pack pro-C18 (150mm, X4.6mm, and 3.0µm) HPLC column, the necessary separations were achieved. For 25 minutes, the developed procedure operated at a 0.6mL/min, flow rate. When determining the NNSI (N-Nitrosamine silodosin impurity) of commercially produced batches of silodosin drug products and drug substances, this established approach can be helpful. The well-known LCMS technique was developed to be selective, Precise, accurate, and Specific.

Keywords: N-Nitroso Silodosin, LOD, LOQ, Sciex-4500 and Silodosin.

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INTRODUCTION

Silodosin is an alpha-1A selective adrenaline activity antagonist used to treat benign prostatic hypertrophy.[1]Because of its high α1A urologic selectivity, silodosin is preferred over other α-blockers for the therapy of LUTS (lower urinary tract symptoms) as well as BPH (benign prostatic hyperplasia).[2]During the synthesis of pharmaceutical drugs, unwanted compounds or impurities are still found in active pharmaceutical ingredients (APIs). Nori, *et al.* Has published reviews regarding nitrosamine impurities in pharmaceutical products and is familiar with regulatory recommendations.[3] The requirement for an analytical technique was created by ICH quality guidelines, specifically chapter number ICH-Q2.[4] The risk of certain approved drug products' mutagenic and carcinogenic potential has grown due to the quantitative presence of n-nitrosamine undesired impurities.[5]Although there have previously been regulatory published reports of N-nitrosamine impurities in the drug products along with drug substances, reports of the finding of NDMA (N-nitrosodimethylamine) in the drug substance Valsartan were published in June 2018 and prompted several regulatory actions, including market recalls and a referral technique under Article 31 of Directive 2001/83/EC in the European Union(EU).[6,7]In the synthetic process of silodosin and degradation procedure of silodosin drug substances, there is a chance of generation of NNSI (N-Nitroso silodosin impurity).An analytical method is needed to determine the content of N-Nitroso silodosin impurity to be in line with the Authority guidelines. This gap has been discovered to affect the quality of silodosin drug substances, and our research starts with a quantitative evaluation of the gap in drug substances by applying the LC-MS methodology.

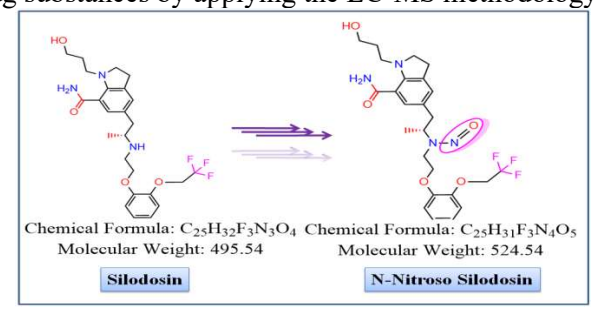

Fig.-1: Schematic Representation for the Formation of N-Nitroso Silodosin Impurity from Silodosin

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EXPERIMENTAL

Materials

LC-MS grade reagents and chemicals were used for the development of the method. Acetonitrile is obtained from Honeywell and Formic acid was obtained from Sigma Aldrich.

Mass Scan Data

Ion Source is selected as ESI (+ve Mode) the ESI Scan of N-Nitroso silodosin was obtained with Sciex-4500 Q-Trap LC-MSMS. The scan data is shown in the Fig.-2.

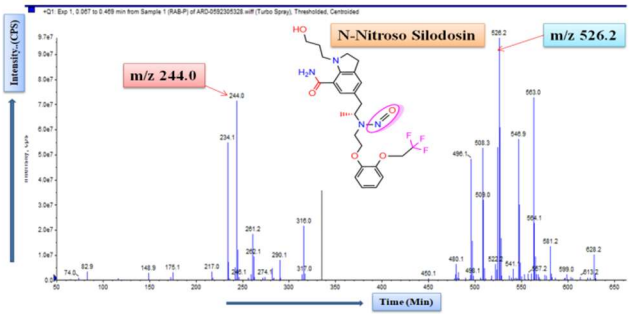

Fig.-2:ESI +ve Scan of N-Nitroso Silodosin

Mobile Phase A

Formic acid (0.1 percent) in Milli-Q-Water (1000µL of formic acid added to the 1liter of Milli-Q-Water).

Mobile Phase B

Acetonitrile: Milli-Q-Water (900:100 v/v)(900mL of acetonitrile along with 100mL of Milli-Q-water are mixed in 1liter mobile phase bottle).

Diluent

Acetonitrile: Milli-Q-Water (50:50 v/v), (500mL of Acetonitrile mixed with 500mL of Milli-Q-Water).

Test Sample Preparation

Weigh accurately test sample (10mg) into a volumetric flask (10 mL), add diluent (5 mL), mix well, make it up to the mark with diluent, as well as filter the mixture using a 0.45µm syringe filter.

N-Nitroso Silodosin Stock Solution Preparation

Weigh NNSI (10.00mg) into the volumetric flask (10mL), then mix diluent (5mL) for dissolving as well as making up the mark with diluent. 0.1 mL of this solution should be poured into the volumetric flask(100mL), where a diluent of 50 ml should be mixed to dissolve as well as make up the difference.

Preparation of Standard Solution (2.25ppm)

Pour 25mL of diluent into a volumetric flask (50mL) containing 0.1125mL of the above N-Nitroso Silodosin stock solution, mix thoroughly, and mark with diluent. This mixture is composed of 2.25 ppm of N-Nitroso Silodosin concerning a sample concentration of 1 mg/mL. The typical mass fragmentation in scan mode and Standard solution chromatogram is shown in Fig.-3.

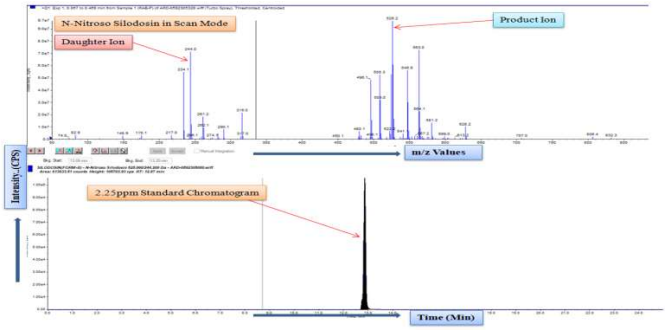

Fig.-3: The Typical Mass Fragmentation in Scan Mode and Standard Solution Chromatogram

RESULTS AND DISCUSSION

Method Development

The process is thoroughly developed using a variety of stationery phase columns that contain amino, PFP, C8, and ascents expressed before it is frozen. These columns are examined using a variety of mobile-phase solutions, including acetic acid, methanol, and ammonium acetate. Ultimately, YMC Pack pro-C18 (150mm, X4.6mm,and 3.0µm) with Formic acid and Acetonitrile with a 25-minute run period yielded the final chromatographic parameters. ESI in +ve MRM mode was used to optimize the mass chromatography.

Chromatographic Conditions:

Flow rate: 0.6mL/min
Injection volume: 20 µL
Column:YMC Pack pro-C18 (150mm, X4.6mm and 3.0µm)
Column oven temperature: 25°C
Sample cooler temperature: 20 °C
Run time: 25min
Diluent: Acetonitrile: Milli-Q-Water (50: 50 v / v)
Mobile phase-A: formic acid (1000µL) in 1Liter of Milli-Q-water
Mobile phase-B: Acetonitrile: Milli-Q-Water (900:100 v/v)
Gradient: Gradient program illustrated inTable-1

Table-1: Gradient Programme

Time	Mobile phase-A	Mobile phase-B
0.00min	80.0%	20.0%
3.00min	80.0%	20.0%
10.00min	50.0%	50.0%
18.00min	10.0%	90.0%
20.00min	10.0%	90.0%
20.10min	80.0%	20.0%
25.00min	80.0%	20.0%

Mass Parameters

Ion Source: ESI (Electro spray ionization)
Scan Type: MRM
Polarity: Positive
Q1 Mass (Da): 525.00 m/z (Product ion)
Q3 Mass (Da): 244.200 m/z value(Daughter ion)
Resolution: Unit
Polarity Dwell time (milliseconds):200
Ion Source gas-1:50
Ion Source gas-2:50
Ion Spray voltage (IS): 4500
Collision gas (CAD): Medium
Temperature: 550°C
CXP (Collision cell exit potential): 6
EP (Entrance Potential): 10

Schematic representation for daughter ions for N-Nitroso silodosin is shown in Fig.-4 and Typical mass fragmentation for N-Nitroso silodosin is shown in Fig.-5.

Method Validation

This recognized approach was verified by ICH-Q2R1 requirements.

System Suitability

N-Nitroso silodosin standard solution (2.25 ppm) was introduced into LC-MS as part of the investigation, and the findings are tabularized in Table-2.

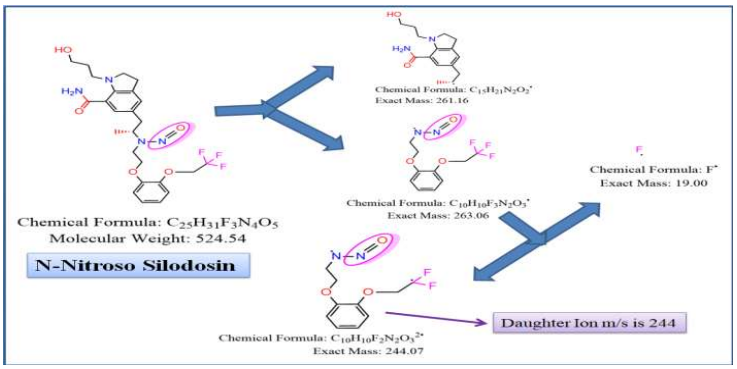

Fig.-4: Schematic Representation of Daughter Ions for N-Nitroso Silodosin

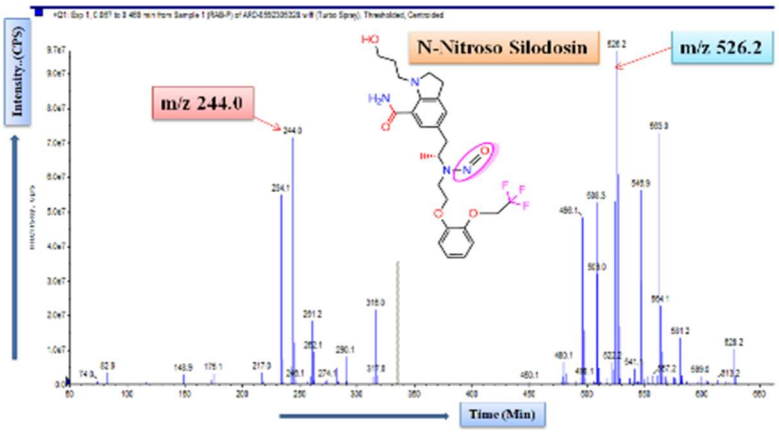

Fig.-5: Typical Mass Fragmentation for N-Nitroso Silodosin

Table-2: System Suitability Data

Preparation	Analyte response
Standard "solution-1	613633.51
Standard solution-2	630389.41
Standard solution-3	613145.43
Standard solution-4	621567.92
Standard solution-5	606593.02
Standard solution-6	616673.09
Average" area	617000.40
STDV	8182.24
% RSD	1.33

The system appropriateness criteria have been verified since the analyte's percentage relative standard deviation (%RSD) area, as determined by six standard solutions, is within the acceptable range.

Specificity

Specificity testing was performed by administering blank, control, and spiked sample solutions. The analysis demonstrated that there was not much interference from the blank solution while the N-Nitroso silodosin peak was retained in the spiked sample solution.

Table-3: LOD and LOQ Data

Name of the Impurity	RT	Concentration (ppm)	S/N at the Specification level	LOD		LOQ	
				S/N	Concentration (ppm)	S/N	Concentration (ppm)
N-Nitroso Silodosin	12.87	2.25	974.88	4.59	0.066	10.24	0.200

LOD and LOQ

The S/N ratio approach was utilized for the determination of LOQ and LOD values as per the ICH Q2R1 guidelines for analytical method validations. The LOD and LOQ values for N-Nitroso silodosin are 0.066 ppm and 0.2 ppm respectively. The % RSD for the peak area of the N-Nitroso silodosin for six LOQ preparations is 2.64. Represented data for LOD and LOQ are mentioned in Table-3, and LOQ precision data is tabulated in Table-4.

Table-4: LOQ Precision Data

Preparation	Analyte response at the LOQ level
LOQ Preparation-1	6118.59
LOQ Preparation-2	6347.54
LOQ Preparation-3	6289.86
LOQ Preparation-4	6014.81
LOQ Preparation-5	6443.05
LOQ Preparation-6	6381.76
Average	6265.94
Standard Deviation	165.40
% RSD	2.64

Accuracy at LOQ Level

Accuracy was established by injection of a preparation of 0.2 ppm analyte (N-Nitroso silodosin) LOQ solution spiked to the test sample in triplicate. The % RSD values obtained were within the acceptance criteria. The outcomes of the accuracy at LOQ level solution analysis are illustrated in the Table-5.

Table-5: Accuracy at LOQ Level Solution (0.2ppm)

Name of the Impurity	Analyte response in LOQ Solution	Analyte response in the test sample	Analyte response in sample + LOQ Solution Spike	Recovery (%)
preparation-1	6118.59	857.20	7836.04	114.06
preparation-2	6347.54	878.60	7568.13	105.39
preparation-3	6289.86	843.58	7789.96	110.44
Average	6252.00	859.79	7731.38	109.96
STEDV	119.08	17.65	143.24	4.36
%RSD	1.90	2.05	1.85	3.96

Accuracy at the Specification Level

Accuracy was established by injection of a preparation of 2.25 ppm analyte (N-Nitroso silodosin) Specification level solution spiked to the test sample in triplicate. The % RSD values obtained were within the acceptance criteria. The results of the accuracy at Specification level solution analysis are shown in Table-6.

Table-6: Accuracy at Specification Level Solution (2.25ppm)

Name of the Impurity	Analyte response in Standard Solution	Analyte response in the test sample	Analyte response in sample + Standard Solution Spike	Recovery (%)
preparation-1	613633.51	857.20	634306.01	103.23
preparation-2	630389.41	878.60	634868.82	100.57
preparation-3	613145.43	843.58	581459.55	94.69
Average	619056.12	859.79	616878.13	99.50
STEDV	9817.95	17.65	30674.68	4.37
%RSD	1.59	2.05	4.97	4.39

Depending on the outcomes of quantitative method evaluation research, the recommendations of the standard test. The procedure for determining the N-Nitroso silodosin in drug substances of silodosin is shorter in Table-7.

Table-7: Method Outcome

Retention time (min)	LOD (ppm)	LOQ (ppm)
12.87	0.066	0.2

CONCLUSION

Developed LC-MS / MS methodology for determination of the trace levels detection of N-Nitroso silodosin in the silodosin drug substance. Depending on the result, this method is specific, sensitive, precise, and accurate. Therefore, the LC-MS/MS methodology may be used for monitoring N-nitroso silodosin and evaluating the standard of the drug substance silodosin. This LC-MS quantitative method has practical advantages for the generic drug industry, helping to maintain the quality and safety of drug substances in silodosin.

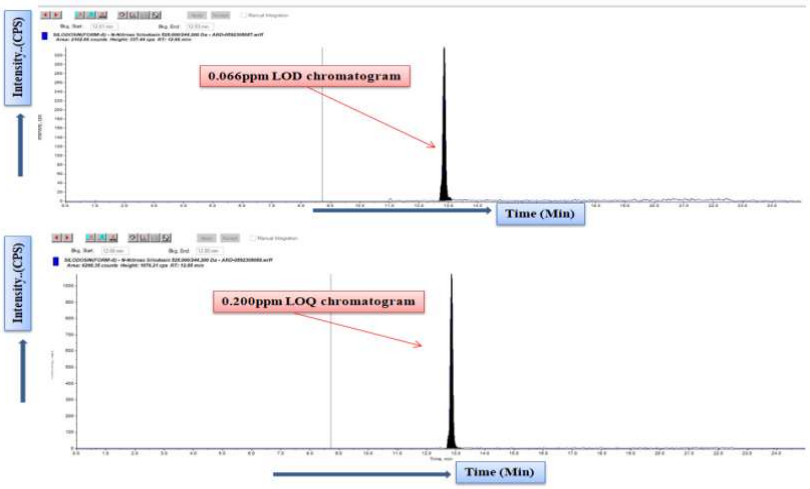

Fig.-6: Typical Chromatogram for LOD and LOQ

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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