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Quantitation of N-nitroso propranolol (NOP) in Propranolol formulation and its placebo using LC-MS/MS

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1. Overview

The potential for N-nitrosamine impurities in pharmaceutical products presents a challenge for the quality management of medicinal products. N-Nitrosamines are considered cohort-of-concern compounds due to the potent carcinogenicity of many of the structurally simple chemicals within this structural class. In the past 4 years, several drug products containing certain active pharmaceutical ingredients (APIs) have been withdrawn or recalled from the market due to the presence of carcinogenic low-molecular-weight N,N-dialkylnitrosamine impurities.

2. Introduction

In 2021, United States Food and Drug Administration (USFDA) received reports of certain types of nitrosamine impurities that formed in several drug products. These nitrosamine drug substance-related impurities (NDSRIs) are a class of nitrosamines sharing structural similarity to the active pharmaceutical ingredients (API). NDSRIs can be generated during manufacturing or during the shelf-life storage period of the drug product and substances. Degradation of APIs, especially those APIs that possess intrinsic reactivity (eg, presence of nitro-alkyl, oxime) or other functionality, or by the presence of an exogenous nitrosating agent, eg, nitrite, is also an important consideration.[1] One such example is N-nitroso propranolol (NOP) in Propranolol API. Propranolol, a synthetic amino alcohol, is a competitive nonselective, β-adrenoreceptor antagonist extensively used to treat hypertension, angina pectoris and other cardiac diseases.[2] β-adrenergic blocking drugs such as propranolol, contain amine groups as a result, these drugs react with sodium nitrite in a hydrochloric acid solution to produce N-nitrosamines.[3] Studies have suggested that genotoxic potency of NOP is 16 folds higher than N-Nitrosodimethylamine.[4] Hence, it is imperative to determine NOP at very trace levels. Structural similarity with API and high carcinogenic potency poses great difficulties for development of low-level analytical methods. This poster demonstrates an LC-MS/MS method for trace level determination of NOP in Propranolol API using an Ultra High Performance Liquid Chromatograph (UHPLC) Nexera™ X3 coupled with an LCMS-8060NX, a Triple Quadrupole Mass Spectrometer from Shimadzu Corporation, Japan (Figure 1).

3. Methods

3-1. LC-MS/MS analysis

Standard for NOP was procured locally. Further, steps such as precursor ion selection, Multiple Reaction Monitoring (MRM) optimization at different Collision Energies (CE) and voltage optimization were performed using Shimadzu's LabSolutions auto MRM optimization feature to obtain MRMs and their optimum CEs. (Table 2) An LC method (Table 1) was developed with an aim to separate the NDSRI, API as well as the excipients, placebo from formulation under study which was achieved using Shimadzu make Shim-pack Scepter C8, 150 mm x 3.0 mm I.D. and 5 µm column. For quantitation, a six-point linearity ranging from 10.0-4000.0 ppb were plotted. The limit of detection (LOD) & limit of quantitation (LOQ) were determined based on S/N & repeatability and were found to be 2.0 and 10.0 ppb, respectively. The S/N at LOD-LOQ, % RSD at LOQ and coefficient of determination (r^2) are shown in Table 2.

Figure 1: Shimadzu Nexera™ X3 UHPLC coupled with an LCMS-8060NX Triple quadrupole

3-2. Analytical conditions

Table 1. Instrument parameters for LC-MS/MS

HPLC System	: Nexera™ X3
Column	: Shim-pack Scepter C8-120, 5 µm 3 x 150 mm (P/N :227-31039-04)
Column Oven	: 40 ° C
Mobile Phases	: A-0.1% Formic acid in LC-MS grade water B-0.1% Formic acid in LC-MS grade methanol
Flow Rate	: 0.5 mL/min
Gradient program (B%)	: 0-3 min → 50 (%); 3-8 min → 50-100 (%); 8-13 min → 100 (%); 13-13.5 min → 100-50 (%); 16 min → STOP.
Injection Volume	: 30 µL
Diluent	: LC-MS grade water: LC-MS grade acetonitrile (1:1 v/v)
LCMS System	: LCMS™-8060NX
Ionization source	: APCI
LC-MS Temperatures	: Interface: 350° C Desolvation Line: 200° C Heater Block: 200° C
LC-MS Gas Flows	: Nebulizing Gas: 3 L/min Drying Gas: 5 L/min
Divert valve program	: 0-6 min to waste; 6-9 min to MS

Table 2: MRM transitions for NOP

Compound	Type	Precursor m/z	Product m/z	CE
NOP	Quantifier	288.85	72.15	-12
	Qualifier	288.85	259.15	-6

3-3. Sample/spiked sample preparation

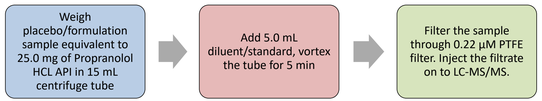

Figure 2 depicts chromatographic overlay of diluent blank, formulation sample, placebo sample and placebo spiked sample at 10 ppb

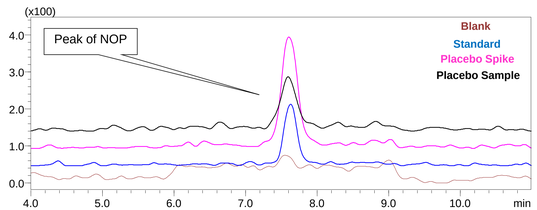

Figure 2 Chromatographic overlay of blank, placebo sample and placebo spiked sample

4. Results and Discussion

Figure 3 depicts the calibration curve, 2.0 ppb and 10 ppb standard of NOP

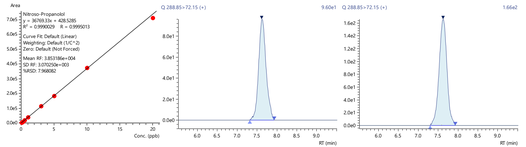

Figure 3: a) Calibration curves b) Chromatograms of 2.0 ppb and 10.0 ppb for NOP

Table 3: Coefficient of determination for calibration curve (CC), repeatability of area ratios for LOQ solution and S/N ratio for LOQ solution (Conc. expressed are relative to sample)

Name	r^2	CC Range (ppb)	LOD Conc. (ppb)	LOQ	
				Conc. (ppb)	% RSD (n=6)
NOP	0.999	10.0-4000.0	2.0	10.0	6.5

Table 4: Summary of NOP concentrations determined in formulation and placebo samples (Conc. expressed are relative to sample)

Concentration of NOP in Propanolol HCL Placebo & Formulation samples (ppb)			
Placebo-1	Placebo-2	Formulation -1	Formulation-2
9.0	16.3	3967.9	3640.6

Both the formulation samples showed presence of higher concentration of NOP, hence placebo sample-1 was used to demonstrate the recovery study. The amount in sample, amount obtained, amount spiked & % recoveries for NOP spike in placebo sample at 10 and 20 ppb are shown in Table 5 and 6.

Table 5: 10 ppb Spiked sample summary

% Recovery of NOP in placebo-1 sample @ 10 ppb				
Amt. in sample (ppb)	Amt. in Spike (ppb)	Amt. found in spike (ppb)	Amt. spiked (ppb)	% Accuracy
9.0	19.3	10.4	10.0	104

Table 6: 20 ppb Spiked sample summary

% Recovery of NOP in placebo-1 sample (@ 20 ppb)				
Amt. in sample (ppb)	Amt. in Spike (ppb)	Amt. found in spike (ppb)	Amt. spiked (ppb)	% Accuracy
9.4	32.8	23.8	20.0	119

5. Conclusion

- Quantitation of Nitroso propranolol in Propranolol formulation and placebo samples was successfully demonstrated on Shimadzu LCMS-8060NX.
- Both the formulation samples were failing and showed higher concentrations of Nitroso propranolol.
- % Recoveries for Nitroso propranolol in placebo sample were found to be between 80-120 %
- Combination of noise reduction technology and sensitive detector enables trace level determination of Nitroso propranolol.

6. References

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