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Application News

Liquid Chromatograph Mass Spectrometer LCMS-8060NX

Quantitation of Nitroso-Propranolol in Propranolol HCl Formulation and its Placebo Using LC-MS/MS

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User Benefits

- An LC-MS/MS method for the low-level determination of Nitroso-propranolol in Propranolol HCl API and formulation
- A quick easy and reliable method for NDSRI meeting regulatory requirement

■ Introduction

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 In the past 4 years, several drug products containing certain active pharmaceutical ingredients (APIs) have been withdrawn or recalled from the market due to the presence of carcinogenic low-molecular-weight N,N-dialkylnitrosamine impurities.

In 2021, United States Food and Drug Administration (USFDA) received reports of certain types of nitrosamine impurities that are formed in several drug products. These nitrosamine drug substance-related impurities (NDSRIs) are a class of nitrosamines sharing structural similarity to the API (having the API or an API fragment in the chemical structure) and are generally unique to each API. NDSRIs form through nitrosation of APIs (or API fragments) that have secondary, tertiary, or quaternary amines when exposed to nitrosating compounds such as nitrite impurities in excipients and can be generated during manufacturing or during the shelf-life storage period of the drug product and substances[1)]. One such example is N-nitroso propranolol (NNP) 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. Structural similarity with API and high carcinogenic potency poses great difficulties for development of low-level analytical methods for estimation of NNP in Propranolol API or formulation. This application news demonstrates an LC-MS/MS method for trace level determination of NNP in Propranolol API and formulation using an Ultra High Performance Liquid Chromatograph (UHPLC) Nexera X3 coupled with an LCMS-8060NX (Figure 1).

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

■ Experimental

An LC method (Table 1) was developed with the aim to separate the NDSRI, API as well as the excipient and placebo from Nitrosamines under study, which was achieved using Shimadzu make Shim-pack Scepter™ C8, 150 mm x 3.0 mm I.D. and 5 µm column. Standard for NNP 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) For quantitation, a six-point linearity ranging from 10-4000 ppb was plotted. The limit of detection (LOD) and limit of quantitation (LOQ) were determined based on S/N and repeatability and were found to be 2.0 and 10.0 ppb, respectively. The % RSD at LOQ and coefficient of determination (r^2) are shown in Table 2.
(All concentrations mentioned above are relative to sample)

■ Method

Table 1: Analytical conditions

HPLC System	: Nexera™ X3
Column	: Shim-pack Scepter C8-120 (150 mm × 3 mm I.D., 5 µm, 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%)	: 50 % (0-3 min) → 100 % (8-13 min) → 50 % (13.5-16 min) → STOP.
Injection Volume	: 30 µL
Diluent	: LC-MS grade water: LC-MS grade acetonitrile (1:1 v/v)
Needle wash	: Water: acetonitrile (1:1 v/v)
MS	: LCMS-8060NX
Ionization source	: APCI
Polarity	: Positive
LC-MS Temperatures	: Interface: 350 °C Desolvation Line: 200 °C Heater Block: 200 °C
LC-MS Gas Flows	: Nebulizing Gas: 3.0 L/min Drying Gas: 5.0 L/min
Divert valve program	: 0-6 min to waste; 6-9 min to MS

Table 2: MRM transitions for NNP

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

■ Sample Analysis

Weigh placebo/formulation sample equivalent to 25.0 mg of Propranolol HCl API in 15.0 mL centrifuge tube

Add 5.0 mL diluent/standard, vortex the tube for 5.0 min

Filter the sample through 0.22 µM PTFE filter. Inject the filtrate on to LC-MS/MS.

Figure 2 depicts chromatographic overlay of diluent blank, standard, placebo sample and placebo spiked sample at 10 ppb in MRM mode.

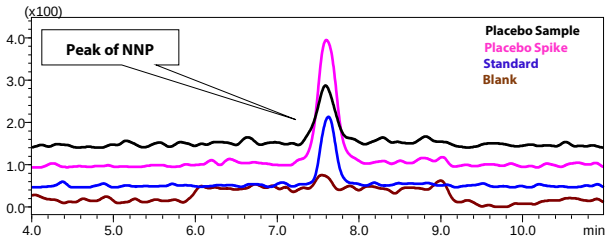

Figure 2: Overlay of MRM chromatograms for blank, placebo sample and placebo spiked sample in MRM mode

■ Results and Discussion

Figure 3 depicts the calibration curve, chromatogram for 2.0 ppb and 10 ppb standard of NNP

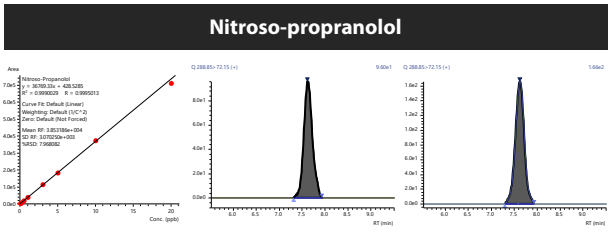

Figure 3: Calibration curve and chromatogram of 2.0 ppb and 10.0 ppb standard solution for NNP

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

Name	r^2	CC Range (ppb)	LOD Conc. (ppb)	LOQ	
				Conc. (ppb)	% RSD (n=6)
NNP	0.999	10-4000	2.0	10.0	6.5

Sample results summary for formulation and placebo samples is tabulated in Table 4.

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

Concentration of NNP in Propranolol HCl Placebo and Formulation samples (ppb)			
Placebo-1	Placebo-2	Sample-1	Sample-2
9.0	16.3	3967.9	3640.6

Both the formulation samples showed presence of high concentration of NNP; hence placebo sample-1 was used to demonstrate the recovery study. The amount in sample, amount obtained, amount spiked and % recoveries for NNP spike in placebo sample at 10.0 ppb are shown in Table 5.

Table 5: Spiked sample summary (10.0 ppb)

% Recovery of NNP in placebo-1 sample @ 10.0 ppb			
Amt. in sample (ppb)	Amt. in Spike (ppb)	Amt. spiked (ppb)	% Accuracy
9.0	19.3	10	103

■ Conclusion

- ✓ Quantitation of Nitroso-propranolol in Propranolol HCl formulation and placebo samples was successfully demonstrated on Shimadzu LCMS-8060NX.
- ✓ Both the formulation samples 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.

■ References

1) United State Food and Drug Administration (USFDA), Control of Nitrosamine Impurities in Human Drugs Guidance for Industry, September 2024, Revision 2

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