U.S. FOOD & DRUG ADMINISTRATION

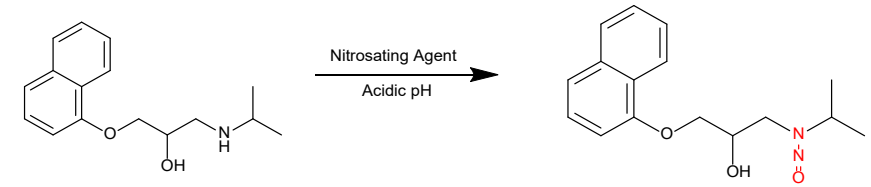
Contains Nonbinding Recommendations

Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS) Method for the Determination of *N*-Nitroso-Propranolol in Propranolol Hydrochloride Oral Solution

This guidance represents the current thinking of the Food and Drug Administration (FDA or Agency) on this topic. It does not establish any rights for any person and is not binding on FDA or the public. You can use an alternative approach if it satisfies the requirements of the applicable statutes and regulations.

Background:

Propranolol is a beta-adrenergic receptor antagonist. The propranolol molecule contains a secondary amine which could form a nitrosamine drug substance-related impurity (NDSRI) when reacted with nitrite under acidic conditions.



Chemical Formula: $C_{16}H_{21}NO_2$
Exact Mass: 259.1572
Molecular Weight: 259.3490

Propranolol

Chemical Formula: $C_{16}H_{20}N_2O_3$
Exact Mass: 288.1474
Molecular Weight: 288.3470

***N*-Nitroso-Propranolol**

To help ensure the safety and quality of propranolol hydrochloride oral solution drug products, the agency has developed and validated a method to determine the presence or absence of *N*-nitroso-propranolol.

Conclusion:

A LC-HRMS method was developed and validated following ICH Q2 (R1) for the detection and quantitation of *N*-nitroso-propranolol in propranolol hydrochloride oral solution. Method verification and/or re-validation is recommended prior to use to demonstrate that the method is suitable for its intended purpose. The limit of quantitation (LOQ), limit of detection (LOD), and range of the method are summarized below:

	***N*-Nitroso-Propranolol**
Limit of Quantitation (LOQ)	0.05 ppm
Limit of Detection (LOD)	0.015 ppm
Range	0.05 - 25.0 ppm

LC-ESI-HRMS Method for the Determination of N-Nitroso-Propranolol in Propranolol Hydrochloride Oral Solution

Purpose

This method is used to detect and quantitate *N*-nitroso-propranolol impurity in propranolol hydrochloride oral solution.

Principle

N-nitroso-propranolol impurity is separated from propranolol hydrochloride by reverse phase chromatography and is detected by a high-resolution and high-mass accuracy (HRAM) mass spectrometer. High sensitivity detection is achieved by monitoring the accurate *m/z* value of the protonated impurity ion. Quantitation is performed by comparing the peak area of the *N*-nitroso propranolol in extracted ion chromatogram (with *m/z* tolerance of ± 15 ppm) of the samples, to the peak area of the *N*-nitroso propranolol reference standard in an external standard calibration.

Reagents

N-Nitroso-Propranolol Reference Standard
Methanol, LC/MS grade
Water, LC/MS grade or equivalent
Formic Acid, LC/MS grade

Equipment/Instrument

UHPLC system equipped with temperature-controlled autosampler and column compartment
Q Exactive orbitrap mass spectrometer (Thermo-Fisher Scientific) or equivalent
HPLC column: Avantor ACE Excel C18-AR, 100 Å, 3 µm, 100 x 4.6 mm (Part # EXL1191046U or equivalent)
Analytical Balance
Vortex Mixer
HPLC vials

Mobile Phase A: Water, 0.1% Formic Acid

Mobile Phase B: Methanol, 0.1% Formic Acid

Diluent: Water

Stock Standard Preparation (100 µg/mL)

Accurately weigh 10 ± 3 mg of *N*-nitroso propranolol reference standard and transfer into a 100 mL volumetric flask. Dilute to volume with ***methanol*** and mix using a stir bar and plate until dissolved.

Intermediate Stock Standard A (200 ng/mL)

Transfer the appropriate aliquot volume of the stock standard into a 250 mL volumetric flask to get a target concentration of 200 ng/mL. Dilute to volume with diluent. Prepare fresh daily.

Intermediate Stock Standard B (10 ng/mL)
Transfer 0.5 mL aliquot volume of the intermediate stock standard A into a 10 mL volumetric flask and dilute to volume with diluent. Prepare fresh daily.

Working Standard and QC Standard preparation (0.5 ng/mL)
Transfer 0.5 mL aliquot volume of the intermediate stock standard B into a 10 mL volumetric flask and dilute to volume with diluent. Prepare fresh daily.

Drug product sample preparation
Dilute the propranolol hydrochloride oral solution with water to obtain a target analytical concentration of 2 mg/mL as propranolol. Mix thoroughly using a vortex mixer. Transfer the sample solution into an HPLC vial for LC/MS analysis.

Chromatographic Conditions

HPLC Column	Avantor ACE Excel C18-AR, 100 Å, 3 µm, 100 x 4.6 mm (Part # EXL1191046U or equivalent)		
Column Temp.	40 °C		
Flow Rate	0.5 mL/min		
Mobile Phase A	Water, 0.1% Formic Acid		
Mobile Phase B	Methanol, 0.1% Formic Acid		
Gradient	**Time (min)**	**A%**	**B%**
	0	50	50
	1.0	50	50
	10.0	0	100
	12.0	0	100
	12.1	50	50
	16.5	50	50
Injection Volume	20 µL		
Autosampler Temp.	10 °C		
Needle Wash	80:20, Methanol:Water, 0.1% Formic Acid		

Mass spectrometer conditions
Instrument
Q Exactive mass spectrometer (Thermo-Fisher)

ESI Ion Source Settings

Sheath Gas Flow Rate	50 arbitrary units
Aux Gas Flow Rate	15 arbitrary units
Sweep Gas Flow Rate	0 units
Spray Voltage	3.5 kV
RF Lens	50
Ion Transfer Tube Temp	250 °C
Vaporizer Temp	400 °C

Scan Settings

Parameters	Q Exactive
Scan Type	PRM
Polarity	Positive
Scan Start – End (min)	9.5 – 12.5
Isolation Window	1.0 *m/z*
Microscans	3
Resolution	70,000
AGC target	1 e^6
Maximum IT	200 ms

Inclusion List

Formula	Adduct	Mass (*m/z*)	Polarity	Start (min)	End (min)	NCE
$C_{16}H_{20}N_2O_3$	+H	289.1547	Positive	9.5	12.5	10

Injection Sequence

Inject Blank (use diluent) at least once at the beginning of a sequence
Inject the Working Standard six consecutive times
Inject the QC Standard before injecting any samples
Inject the QC Standard once every six injections of the samples and at the end of a sequence.

Example:

Order	Solution	No. of Injections
1	Blank	2
2	Working Standard	6
3	QC Standard	1
4	Blank	1
5	Sample 1	1
6	Sample 2	1
7	Sample 3	1
8	Sample 4	1
9	Sample 5	1
10	Sample 6	1
11	QC Standard	1
…	…	…

System Suitability

The % RSD (n = 6) of the *N*-nitroso propranolol impurity peak areas for the first six injections of the working standard solution should not be more than 10%.
The % recovery of the QC Standard should be between 80 – 120%.
The overall % RSD of the *N*-nitroso propranolol impurity peak areas from the combined working standard and QC standard solution injections should not be more than 20%.

Data Processing

N-nitroso-propranolol impurity peak areas from the extracted ion chromatograms (EIC) with a *m/z* tolerance of ±15 ppm are used for quantitation. The *N*-nitroso-propranolol *m/z* value to be extracted is listed below:

***N*-Nitroso-Propranolol**	
m/z to be extracted for quantitation	72.0813

The retention time difference of the *N*-nitroso-propranolol peak in the analyzed samples should not be more than 2% of the retention time of the corresponding *n*-nitroso propranolol peak in the reference standard solution.

Calculation:

$$N\text{-nitroso-Propranolol (ppm)} = \frac{A_{spl}}{As} \times C_s \times \frac{1\ mg}{1\times10^6\ ng} \times \frac{1}{2\ mg/mL} \times 10^6$$

where: A_{spl} = Area of the *N*-nitroso-propranolol peak in the sample solution
As = Average area (n = 6) of the *N*-nitroso-propranolol peak from the first six consecutive injections of the working standard
C_s = Concentration of the *N*-nitroso-propranolol in the standard solution (ng/mL)