

# Development and Validation of an Ultra Sensitive UPLC-MS/MS Method for the Determination of Naloxone in Human Plasma

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## Introduction

Naloxone(NLX) is a  $\mu$ -opioid receptor antagonist and commonly used for counteracting opiate overdose. The combination use of buprenorphine and low dose of NLX was approved by the FDA to prevent illicit intravenous use of this formulation. The low dose of NLX requires a corresponding low limit of quantitation of NLX for pharmacokinetic studies (i.e. 5 pg/mL). Naloxone-3-glucuronide(NLG) is a major metabolite of NLX. The instability of NLG can impact the accurate measurement of NLX at such a low quantitation limit. This poster will summarize development and validation of an ultra sensitive LC-MS/MS assay to quantify NLX in human plasma without the impact from NLG. The assay is five times more sensitive than a recently published LC-MS/MS method for NLX.

## Methods

The sample extraction of NLX from human plasma employed the polymeric cation exchange solid-phase extraction using Statra X-CW 96-well plate. The UPLC-MS/MS analysis was performed on a Shimadzu Nexera system coupled with AB Sciex Triple Quadruple MS 5000. The gradient elution used a Waters UPLC BEH C18 column and the mobile phase was 0.1% NH<sub>4</sub>OH in water and 0.1% NH<sub>4</sub>OH in methanol. The MS detection in multiple-reaction-monitoring (MRM) mode for NLX was m/z 328.1→212.1 and for the internal standard, NLX-d<sub>5</sub>, was m/z 333.1→212.1. The total analysis time was 4 min. The linearity, accuracy, precision, selectivity, dilution integrity, and stability of this method were validated.

## Preliminary Data

The LC separation was performed under basic condition to better retain NLX and achieve symmetrical peak shape. Based on the chemical property of NLX, the SPE procedure was first optimized with Phenomenex X-C plate as a strong cation exchange phase to achieve high recovery of 94%. However, the X-C eluents yielded high baseline, making it difficult to achieve adequate S/N at LLOQ level. The SPE was then re-developed using Phenomenex X-CW plate as a weak cation exchange phase. With comparable recovery, X-CW eluents gave 5 times' lower baseline and less matrix suppression (5.6%) when compared to that given by the X-C sorbent (33.2%). The X-CW SPE wash and elution solvents were further optimized to achieve LLOQ of 5 pg/mL with a sample volume of 350  $\mu$ L. To investigate the stability of NLG, the benchtop incubation was conducted at both room temperature and 4 °C. The results showed that the conversion from NLG to NLX was 23.0% after 24 hour storage at room temperature but was insignificant at 4 °C. In addition, the conversation of NLG was monitored during the extraction and post-extraction storage to ensure no impact on accurate determination of NLX in the presence of NLG. The results showed no conversion of NLG for at least 5 days storage in the reconstitution solvent of methanol:water/5:95 (v:v). Over a linear range of 5-500 pg/mL, the intra-day and inter-day accuracy and precision were within 6.8% bias and 6.9% RSD, respectively. The stability of NLX was also

evaluated to ensure it was stable for at least 2 hours in whole blood and 24 hours in human plasma at 4 °C.

**Novel Aspect**

An ultra sensitive UPLC-MS/MS method has been developed to quantify naloxone without interference from a labile major glucuronide metabolite.