Development and validation of an LC-MS/MS method for screening and quantification of trace N-nitrosamines in a pharmaceutical formulation

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1. Introduction

Organic compounds such as **N-nitrosamines** (NAs) can occur naturally or be formed in the environment and food. Many of those have been identified as DNA-reactive mutagens and could lead to cancer.

In recent years, significant amounts of NA impurities have also been found in pharmaceuticals for human use, raising serious health concerns. Both EMA and FDA mandate manufacturers to evaluate, investigate, and control the presence of NAs in medicinal products.

This work aimed to develop a highly specific and sensitive analytical method coupling **reverse phase liquid chromatography (LC) with tandem mass spectrometry detection** for the exploratory phase to determine four trace impurities of NAs simultaneously.

2. Material & Method

An Acquity Premier LC system (Waters, USA) was used for NAs separation and hyphenated to an ultra-sensitive Xevo TQ-Absolute tandem mass spectrometer (Waters, USA) equipped with an **APCI** source operating in **positive polarity** for detection.

Four NAs were specifically selected in the context of the Quality Control of a commercialized pharmaceutical product.

The method was developed using an innovative ***in-silico*** **approach** allowing flexibility in terms of NAs targeting and **matrix challenges** to be solved. Validation of the method was conducted under the recommendations of **ICH Q2(R1)** using the **accuracy profile** approach. At least four validation series should be performed.

3. Results

3.1. In silico QSRR modeling

Separation criterion: NAs separated from the API to reduce matrix effect (ME)

Optimal predicted conditions:
- pH 2.7
- tG 20 min

Geometric transfer

tG 9.9 min

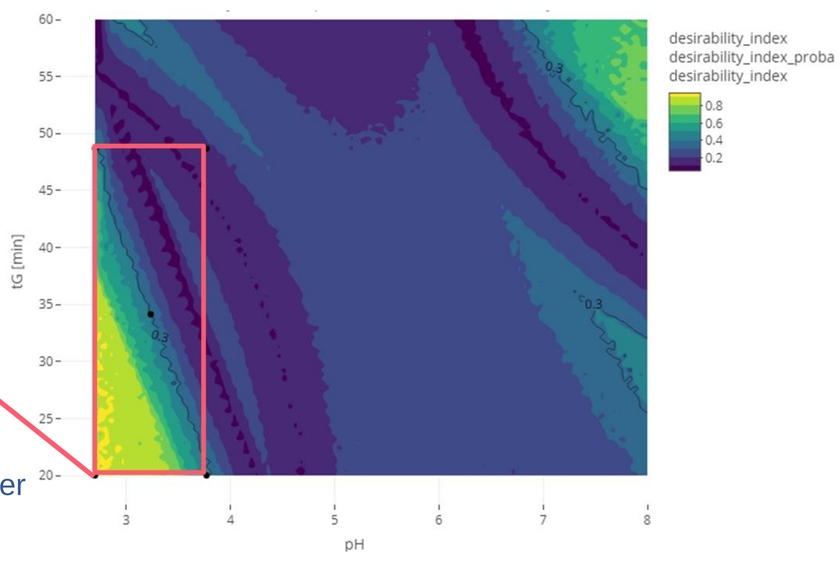

Figure 1. *In-silico-assisted screening of gradient time (tG) and pH conditions for optimal LC separation between 4 NAs.*

3.2. Experimental LC separation

NNK and API have close retention times under predicted LC conditions. NNK is affected by the ME, resulting in ionization suppression due to co-elution with API. The other NAs are sufficiently separated from the API.

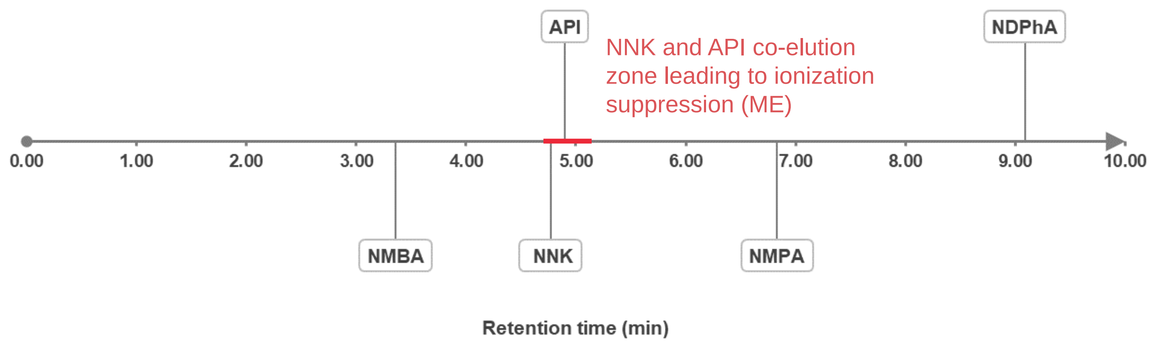

Figure 2. *Experimental chromatographic separation of NAs under predicted LC and optimized MS/MS conditions.*

3.3. Validation – limit test

For all NAs, selectivity, specificity and LoD were validated as an impurity limit test. Only the results for NMBA and NNK are presented below.

LoD was estimated by extrapolating the average S/N ratio obtained at 0.25 ng/mL from the quantifier ion over all the validation series to the reporting threshold (≥3).

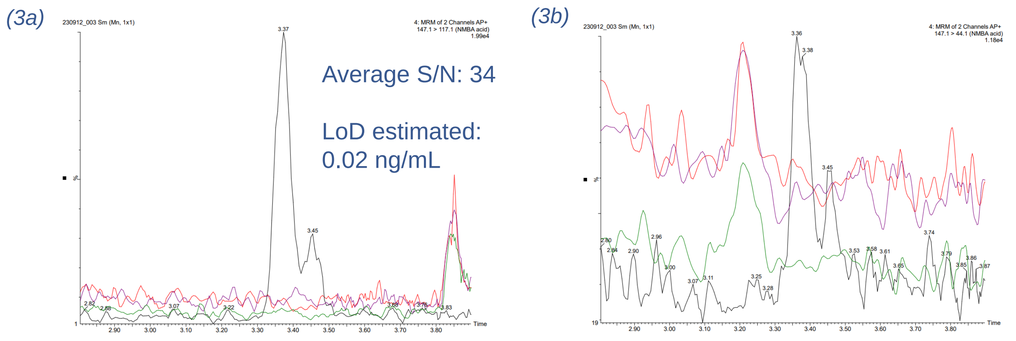

Figure 3. *NMBA at quantifier (3a) and qualifier (3b) transitions – overlayed extracted ion chromatograms (black) Standard solution at 0.25 ng/mL; (green, purple, red) 3 independent matrices*

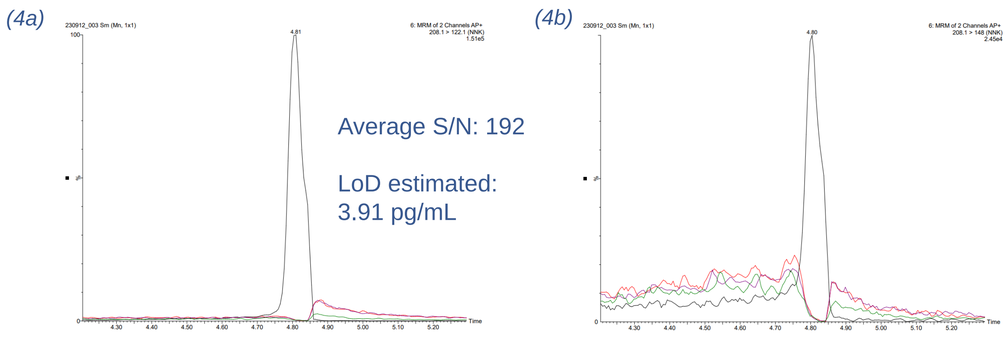

Figure 4. *NNK at quantifier (4a) and qualifier (4b) transitions – overlayed extracted ion chromatograms (black) Standard solution at 0.25 ng/mL; (green, purple, red) 3 independent matrices*

3.4. Validation – assay

The study was expanded to validate the assay for NMPA and NDPhA. Quantitative performances were assessed by considering the total error of the method using the accuracy profile approach.

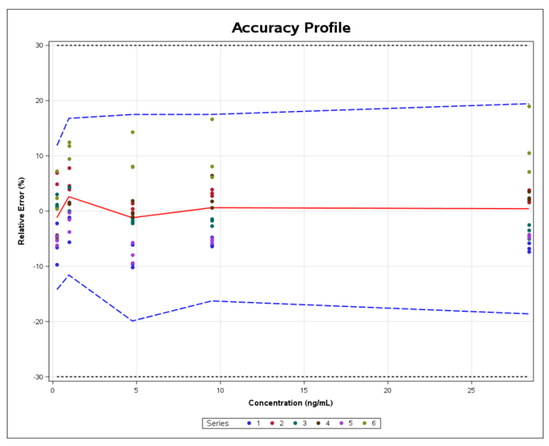

Figure 5. *Accuracy profile for NMPA quantification in the formulation considering the response function "Linear regression Through 0. Fitted using level 3 only". Plain red line: relative bias; dashed blue lines: β-expectation tolerance limits with an α-risk of 5%; dashed black lines: acceptance limits at ± 30%; dots: Validation standards.*

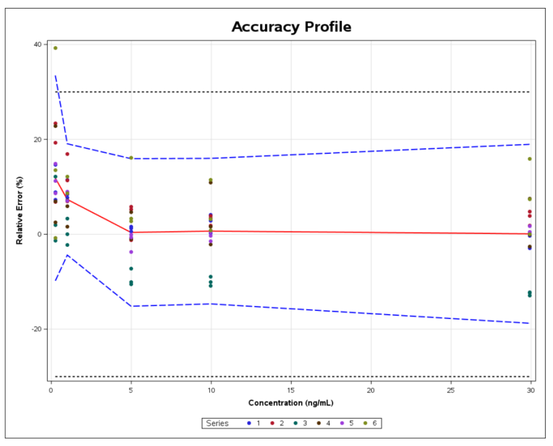

Figure 6. *Accuracy profile for NDPhA quantification in the formulation considering the response function "Linear regression Through 0. Fitted using level 3 only". Plain red line: relative bias; dashed blue lines: β-expectation tolerance limits with an α-risk of 5%; dashed black lines: acceptance limits at ± 30%; dots: Validation standards.*

4. Conclusions

This work highlighted the possibility of using QSRR modeling to facilitate LC development. This *in-silico* approach could be extended to the other NAs under the EMA's oversight, enabling alternative LC methods to be proposed.

The current method is validated for the 4 N-nitrosamines as a limit test for impurities and as an assay for NMPA and NDPhA. The method demonstrates adequate quantitative performance, with a validated dosing range between 0.5 ng/mL and 30 ng/mL.

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