Analytical Considerations for Nitrosamine Formation when Formulating pMDI's with Next-Generation Low GWP Propellant Systems

John McLaughlin[1], Mark Parry[1] & Miles Jeanneret[1]

[1]Intertek Melbourn, Saxon Way, Melbourn, Herts, SG8 6DN, UK

Summary

The global transition toward environmentally sustainable pressurized metered-dose inhalers (pMDIs) requires a thorough understanding of potential impurities introduced during reformulation, particularly nitrosamines, a class of potential human carcinogens that has seen significant development of legislation and understanding of risk in recent years. This study presents work developing analytical methods and investigating the risk of nitrosamine formation in marketed pMDI pharmaceutical products. Further studies will extend this work to look at the risks and control strategies when undergoing reformulation with next-generation low Global Warming Potential (GWP) propellants.

Two commercially available pMDIs, Symbicort, containing formoterol, and Ventolin, containing salbutamol, were assessed for the formation of their respective nitrosamine impurities, N-nitroso formoterol (NNF) and N-nitroso salbutamol (NNS). Sensitive LC-MS methods were developed for both, achieving limits of quantification well below established acceptable daily intake (ADI) thresholds. Linearity and accuracy were confirmed across relevant concentration ranges. Initial testing showed trace levels of NNF near the method's limit of detection in Symbicort, while no NNS was detected in Ventolin. Following forced degradation at 60 °C for up to six weeks, Symbicort exhibited quantifiable levels of NNF, though still significantly below the ADI, while Ventolin remained nitrosamine-free throughout.

This proof-of-concept work demonstrates the utility of the analytical methods and supports their application in ongoing studies. Future work will evaluate nitrosamine formation following incorporation of low GWP propellants and examine interactions with device components (e.g., valves, seals, actuators) to identify potential sources of nitrosamine formation.

These findings will guide safe, compliant reformulation strategies, ensuring continued patient access to essential inhalation therapies with a lower environmental impact.

Key Message

Robust LC-MS methods have been developed to detect trace-level nitrosamines in pMDIs, providing a critical analytical foundation to evaluate and control nitrosamine risks during reformulation with low GWP propellants, supporting regulatory compliance, sustainable, and environmentally friendly inhaler development.

Introduction

Since the EMA and FDA introduced guidance in 2020 to control nitrosamine impurities in medicinal products, pharmaceutical developers have invested significant resources into risk assessment, analytical method development, and root cause investigations. These efforts are essential but resource-intensive, often requiring complex reformulation to meet stringent acceptable intake limits.

The planned transition to low Global Warming Potential (GWP) propellants in pMDIs presents an important opportunity to proactively address nitrosamine formation risks early in formulation development. This is particularly crucial, as pMDIs contain multiple potential sources for nitrosamine generation, including interactions among propellants, APIs, excipients, and device components such as valves and elastomers.[1]

This study established sensitive, selective LC-MS methods for detecting trace-level nitrosamines in commercial pMDIs, with quantification capabilities well below regulatory thresholds. The nitrosamines of interest, NNF in Symbicort and NNS in Ventolin, were selected due to the active ingredients, formoterol and salbutamol, containing secondary amines that are susceptible to nitrosation. Both share an ADI limit of 1,500 ng/day.[2] Based on the respective product dosing regimens, this equates to in-vial nitrosamine concentrations of 900 ng/mL for Symbicort and 750 ng/mL for Ventolin.

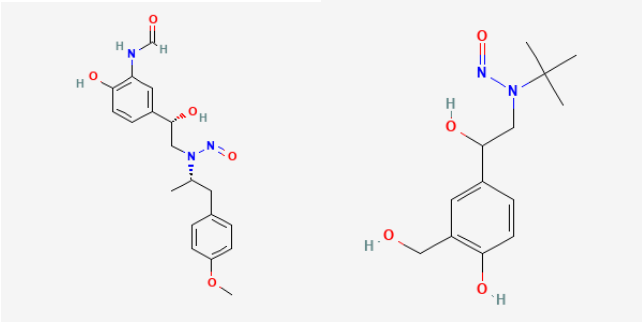

Figure 1 - Structures of NNF[3] (Left) and NNS[4] (Right)

These methods have been successfully applied in initial forced degradation studies to simulate worst-case storage conditions, forming a proof-of-concept foundation for future risk assessment. As next-generation low GWP propellants like HFO-1234ze and HFA-152a are introduced, their distinct chemical properties may lead to new degradation pathways and require re-evaluation of formulation and component materials. Their reactivity under thermal or humid conditions—and potential influence on nitrosamine formation—remains poorly understood. [5] This work provides a critical first step in assessing those risks and guiding the safe, compliant, and environmentally responsible reformulation of pMDIs.

Materials and Equipment

Analysis was performed using a Thermo Vanquish LC coupled to a Thermo TSQ Quantis mass spectrometer, with reverse-phase chromatography and electrospray ionisation in negative mode. Optimised MRM transitions were 372.2 → 175.0 for NNF and 267.0 → 151.0 for NNS. Separation for NNF was achieved using a Waters Acquity BEH C18 column (2.1 × 100 mm, 1.7 µm), and for NNS using an XSelect HSS T3 column (3.0 × 100 mm, 3.5 µm), with flow rates of 0.4–0.6 mL/min and injection volumes of 10–25 µL. Calibration was performed using external standard solutions.

Sensitive LC-MS methods were developed to detect trace levels of NNF and NNS in Symbicort and Ventolin, respectively. The methods achieved LOQs well below the 1,500 ng/day ADI and demonstrated suitable linearity, accuracy, and precision for trace-level monitoring in pMDIs.

Symbicort and Ventolin canisters were prepared by freezing at –80 °C, then cutting open while the propellant remained in the liquid phase. This allowed controlled evaporation, leaving behind the formulation residue, which was quantitatively rinsed into a 50 mL volumetric flask, filtered, and prepared for analysis.

Results and Discussion

The developed LC-MS methods demonstrated excellent sensitivity, with LOQs of 0.4 ng/mL for Symbicort and 0.1 ng/mL for Ventolin. These values correspond to daily intakes of approximately 0.66 ng/day and 0.32 ng/day, respectively, both several orders of magnitude below the ADI's of 900 ng/mL for Symbicort and 750 ng/mL for Ventolin. LODs were also determined to support trace-level detection and early identification of low-level nitrosamine formation. These are summarised in Table 1.

Table 1 Summary of Testing Limits

Criteria	Symbicort	Ventolin
Testing Limit per day	1500ng/day	1500/day
Required Nitrosamine Testing limit (in solution)	**900 ng/mL**	**750 ng/mL**
Method LOD (in solution)	0.03 ng/mL	0.12 ng/mL
Method LOQ (in solution)	0.1 ng/mL	0.4 ng/mL
Method Testing Limit per day	**0.17 ng/day**	**0.8 ng/day**

Linearity was confirmed for both methods across ranges sufficient to quantify the observed nitrosamine levels and are therefore set significantly lower than the ADI limit. Correlation coefficient R^2 values >0.99 were achieved for the ranges examined and representative linearity plots are shown in Figure 2.

Accuracy was evaluated at both the limit of quantification (LOQ) (0.1 ng/mL NNF, 0.4 ng/mL NNS) and the Upper limit of quantification (ULOQ) (10 ng/mL for both NNF and NNS) in triplicate (Tables 2 – 3). Recovery data were overall good for both components, although if higher levels of the nitrosamine were present, optimisation for the exact range needed may improve performance further.

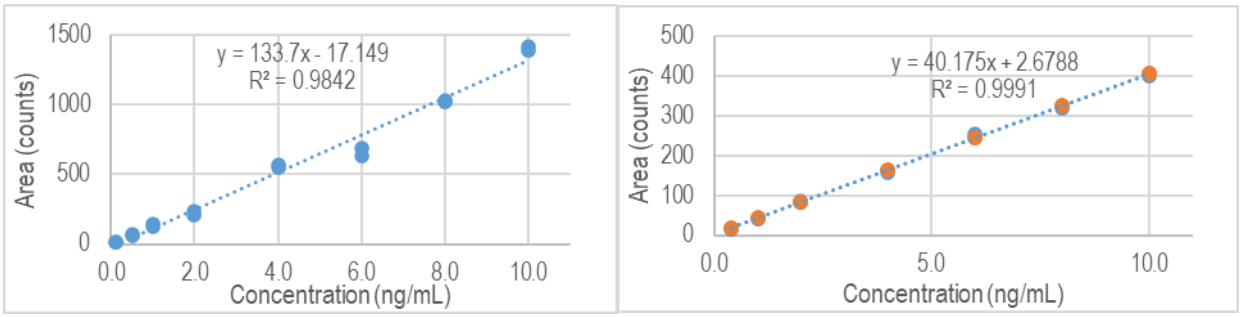

Figure 2 - Linear Regression plots for NNF (Left) and NNS (Right)

Table 2 Summary of Accuracy Results for NNF in Symbicort

Accuracy Level	Recovery %	Mean Recovery %
Symbicort (NNF) 0.1 ng/mL LOQ Rep 1	91.6	103.0
Symbicort (NNF) 0.1 ng/mL LOQ Rep 2	108.2	
Symbicort (NNF) 0.1 ng/mL LOQ Rep 3	109.2	
Symbicort (NNF) 10 ng/mL ULOQ Rep 1	89.5	91.7
Symbicort (NNF) 10 ng/mL ULOQ Rep 2	94.4	
Symbicort (NNF) 10 ng/mL ULOQ Rep 3	91.3	

Table 3 Summary of Accuracy Results for NNS in Ventolin

Accuracy Level	Recovery %	Mean Recovery %
Ventolin (NNS) 0.4 ng/mL LOQ Rep 1	101.9	109.1
Ventolin (NNS) 0.4 ng/mL LOQ Rep 2	113.2	
Ventolin (NNS) 0.4 ng/mL LOQ Rep 3	112.2	
Ventolin (NNS) 10 ng/mL ULOQ Rep 1	114.7	115.4
Ventolin (NNS) 10 ng/mL ULOQ Rep 2	115.3	
Ventolin (NNS) 10 ng/mL ULOQ Rep 3	116.1	

Testing of Marketed Products

Initial testing of in-market samples showed that Symbicort exhibited trace-level NNF just above the LOQ, while Ventolin remained below the LOD, indicating no detectable nitrosamine formation under standard storage conditions (Figure 3).

A forced degradation study was conducted by storing canisters at 60 °C for 11 days and up to 11 weeks to simulate extreme long term thermal stress.

For Symbicort, NNF levels increased above the LOQ following both 11 days and 11-week storage. After 11 days at 60 °C, the concentration was approximately 0.1 ng/mL (0.17ng/day). After 11 weeks, levels rose to approximately 0.5 ng/mL (0.83 ng/day), remaining below 0.05% of the ADI. Representative chromatograms are shown in Figure 3.

Ventolin remained nitrosamine-free throughout all degradation periods, with no quantifiable NNS peaks above background (Figure 3). This suggests formulation or component specific factors may contribute to nitrosamine formation in Symbicort but not in Ventolin.

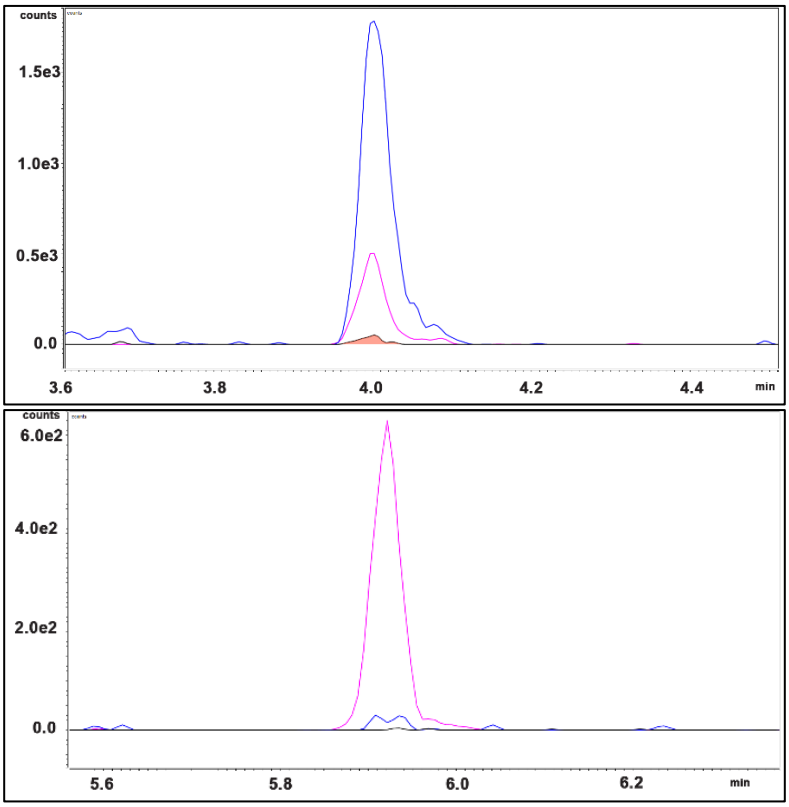

Figure 3 - Chromatogram NNF (Top) and NNS (Bottom). (Black/highlighted pink = Unstressed, blue = stressed and pink = solution LOQ)

Implications for Risk Assessments

The detection of trace NNF in Symbicort, particularly following thermal stress, highlights the importance of early and sensitive analytical monitoring. While levels remained below the ADI, their appearance under stress conditions underscores the need to understand formulation and component-related influences on nitrosamine formation.

The contrast between Symbicort and Ventolin may reflect differences in API chemistry, excipient compatibility, or potential catalytic effects from device components. Additionally, the observed differences in nitrosamine formation rates suggest underlying variations in chemical kinetics between the two compounds, with formoterol potentially more susceptible to nitrosation under stress conditions than salbutamol. These findings support the need for further targeted risk assessments to better understand the contributing mechanisms.

Next Steps and Relevance to Reformulations

This initial work provides a sensitive analytical platform and a baseline understanding of nitrosamine risk in current pMDIs. Future studies will focus on incorporating low-GWP propellants, such as HFO-1234ze and HFA-152a, into test formulations of Symbicort and Ventolin.

These next-generation propellants may introduce new degradation or interaction pathways under conditions of heat, humidity, or contact with device components. Importantly, their distinct chemical properties—such as reactivity, unsaturation, or impurity profiles—could alter the kinetics of nitrosamine formation, potentially accelerating or suppressing nitrosation reactions depending on the formulation context. Planned studies will evaluate these risks through forced degradation and material interaction experiments. Additional work may involve test systems isolating specific pMDI components (e.g., valves, elastomers) to further identify nitrosamine-contributing sources, as part of the reformulation efforts may also bring with them new can and valve parts which may have critical influence regardless of the propellent used.[6]

Conclusion

Sensitive LC-MS methods have been developed to detect nitrosamines in two marketed pMDIs, with LOQs well below regulatory limits. Symbicort showed very low-level NNF formation under thermal stress but not exceeding <0.05% of the ADI, while Ventolin remained nitrosamine-free—indicating potential formulation- or component-specific contributors. These results demonstrate the methods' suitability for trace-level monitoring in pMDIs.

Ongoing work will assess the effects of low-GWP propellants on nitrosamine formation. Forced degradation and material interaction studies will support safe, compliant, and environmentally responsible reformulation of inhaler products.

References

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