LCMS/MS Quantification of N-Nitrosopseudoephedrine in Drug Products using Vitamin E as a Nitrite Scavenger

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Introduction

- N-nitrosamines are a class of impurities with suspected genotoxic and carcinogenic effects on humans.[1] When present in pharmaceutical products, they represent a significant health risk to patients.
- Many Active Pharmaceutical Ingredients (API) contain nitrosatable amine functional groups and are therefore at risk of forming n-nitrosamine drug substance-related impurities (NDSRIs).
- Regulatory authorities require pharmaceutical manufacturers to risk assess all pharmaceutical products for the presence of N-nitrosamines, and where a risk is identified, develop methods to analyze them.
- False positive results can result in product recalls. These have significant financial implications for a pharmaceutical company and may impact patients by reducing the availability of pharmaceutical products.
- High levels of N-Nitrosopseudoephedrine (NPEP) were observed during development of a method for a major pharmaceutical company's drug product. This study describes experiments investigating N-nitrosamine formation and reduction of false positive results via the use of Vitamin E as a nitrite scavenger.

Nitrosamine Formation

- Pseudoephedrine can form NPEP by reacting with nitrosating compounds such as sodium nitrite.
- Sodium nitrite is often already present at acceptable levels in drug products as an impurity in excipients.[3]
- Under acidic conditions, nitrite ions reacts with secondary or tertiary amines to form N-nitrosamines (Figure 1).
- Sub parts per million levels of nitrite in a drug product can potentially result in the formation of N-nitrosamines above allowable daily intake limits set by regulators for NDSRIs.
- Sample solutions spiked with increasing amounts of nitrite resulted in an increase of the determined NPEP levels.
- This demonstrated that the sample preparation conditions were favourable for N-nitrosamine formation and that the result was a potential false positive.

Nitrosamine Inhibition

- A multitude of compounds exist that are capable of scavenging nitrite. These have different physiochemical properties to consider prior to inclusion in an analytical method.[4]
- Vitamin E was selected due to its demonstrated scavenging ability and solubility in organic diluent for drug product dissolution.
- A significant decrease in N-nitrosamine formation was observed upon adding vitamin E into the diluent for sample solution preparation.
- Subsequent optimisation showed that the inhibition of N-nitrosamine formation reached a plateau as the concentration of vitamin E was increased up to 10 mg/mL.

Conclusion

- Mitigating the risk of false positive results during the analysis of N-nitrosamines is critical for preventing unnecessary disruption to the manufacture and distribution of therapeutic products.
- Nitrite spiking experiments were used to detect the formation of N-nitrosamines during sample preparation.
- The use of a vitamin E in the sample diluent as a nitrite scavenger reduced the formation of NPEP during analysis and mitigated the risk of a false positive result.

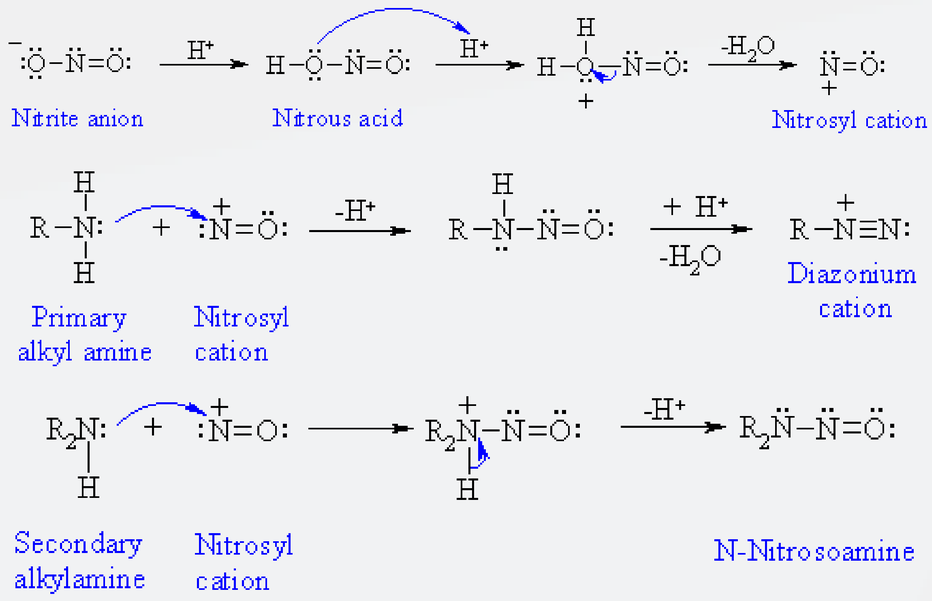


Figure 1: Example formation of nitrosamines from a primary or secondary amine with sodium nitrite.[2]

Figure 2: NPEP Concentration of Drug Product Solutions versus Concentration of Sodium Nitrite

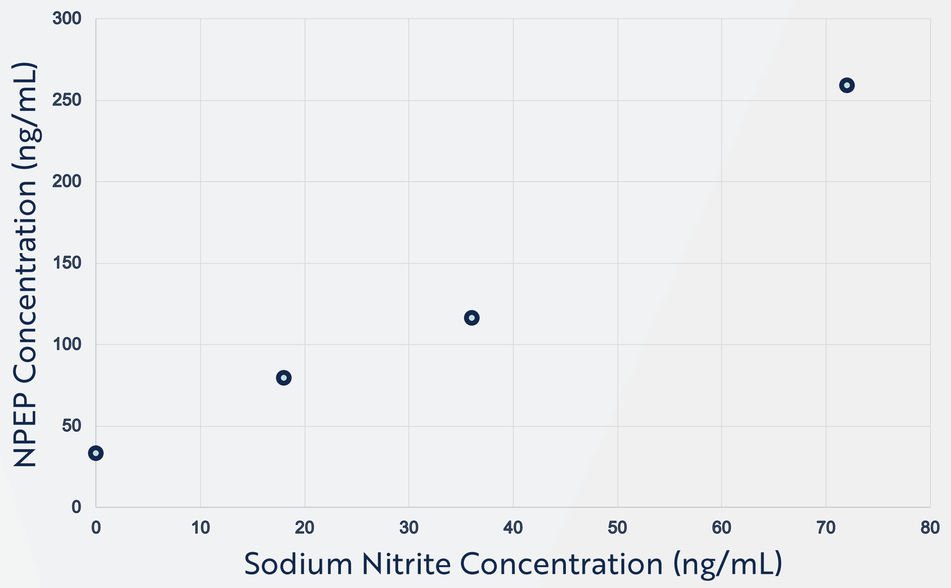
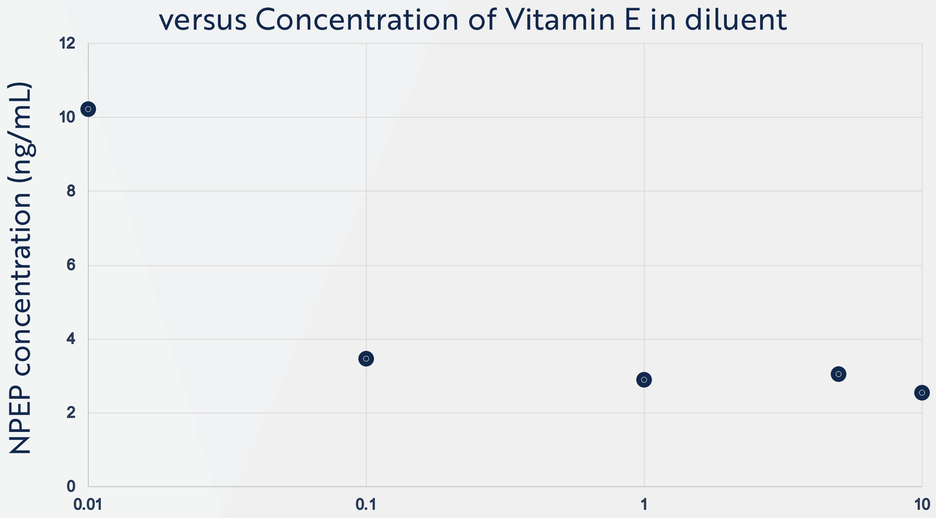


Figure 3: NPEP concentration of Drug Product Solution versus Concentration of Vitamin E in diluent



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