# A Simple and Reliable Technique for Trace Level Quantification of Nitrite as Precursors of N- nitrosation in pharmaceutical samples

## INTRODUCTION:

N-nitrosamines are potent genotoxic agents, and some are classified as probable human carcinogens. N-Nitrosamines are formed when nitrites react with a secondary or tertiary amine especially under acidic conditions.

Most cases of N-nitrosamine contamination can be attributed to the presence of nitrites and relevant amines during a manufacturing process. All processes that use or generate nitrites should be considered high-risk in relation to contamination with N-nitrosamine impurities. Therefore, testing of raw materials, intermediates and excipients for nitrites is a critical step during pharmaceutical production. Nitrites can be found in most excipients at parts per million (ppm) levels . HPLC with mass detection-based method was developed for screening of trace levels of nitrite in excipients, active pharmaceutical ingredients and pharmaceutical formulations. The principle of this method is pre column derivatization which involves the reaction of nitrite ions with 2,3-diaminonaphthalene (DAN) to form 1- [H]-naphthotriazole (NAT) (Fig. 1) which is then separated by HPLC and detected by mass detector

**Fig 2: Arc HPLC with Acquity Qda Dtector**

## SCOPE OF WORK:

Nitrite concentration is the most important factor governing Nitrosation in pharmaceutical samples and Nitrites being precursors of N-nitrosamines, influence the formation of all possible N-nitrosamines. Screening of nitrite precursor using one rapid and highly selective method is significantly less expensive and less time-consuming than screening for NDSRIs. Decreasing the nitrite concentration could eliminate the N-nitrosamine formation. Waters Arc HPLC coupled with Acquity QDa, and X select CSH C18 Column combination produced robust method for quantification of Nitrite residue. The LOQ was defined as the first calibration point, concentration of nitrite corresponding to 0.2 ppm with respect to the placebo, and the signal-to noise ratio (S/N) was found to be about 100. The LOD was evaluated and found to be 0.005 ppm of nitrites relative to sample weight.
The observed spiked recovery was 70 to 120 % by adapting an extraction approach.

| Test | Limit/Range |
|---|---|
| Linearity | 0.2ppm to 20.0 ppm |
| Method LOD | 0.005 ppm |
| Method LOQ | 0.2 ppm |
| Spiked recovery | 70 to 120 % |

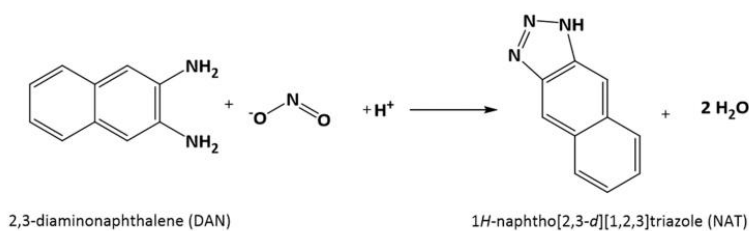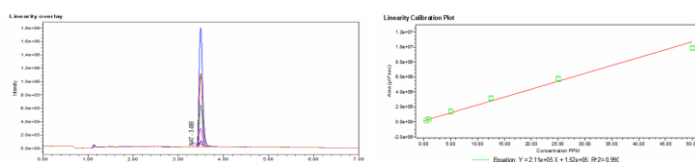**Table 1: Summary for Nitrite content**



**Fig 1: Derivatization reaction of nitrites with 2,3-diaminonapthalene (DAN)**

## Linearity and Precision:

The calibration curve was constructed by plotting the peak area against the concentration (six concentration levels) in the range from 0.2 ppm to 20 ppm (0.2, 2.0, 5.0, 10.0 and 20 ppm). Each calibration reference solution was measured in duplicate. Good linearity was achieved with the correlation coefficient of 0.99.

The method precision of the nitrite content was evaluated by analysis of six replicate injections of the same homogenous sample (n = 6) with three various concentration levels (0.5ppm,1.0ppm and 5ppm) in three different days. The precision was expressed in terms of relative standard deviation (RSD). The results were less than 2% for all three levels.



**Linearity and Precision results**

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