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Letter to the editor of Heliyon re: determination of dimethylamine and nitrite in pharmaceuticals by ion chromatography to assess the likelihood of nitrosamine formation Heliyon. 2021; 77: e06179

ABSTRACT

Our letter to the editor of Heliyon outlines queries on the methodology and sample preparation used in article e06179, published in 2021. The nitrite measurements reported are higher than those observed in our experience. In the interest of reporting nitrite levels that are fully accurate, we would like to discuss the findings with the article authors.

1. Introduction

Nitrosamine risk assessment and control have become an integral part of pharmaceutical drug product development and quality evaluation. Initial reports of nitrosamine contamination were linked with the drug substance and its manufacturing process. Subsequently, the drug product and aspects of the formulation process have shown to be relevant, including the presence of nitrite in excipients and the active ingredient. Accurate knowledge on the presence and distribution of nitrite in excipients has become an important goal, both to pharmaceutical manufacturers and health authority regulators, as it is a key piece of information used in the risk assessment for nitrosamine formation in the drug product. Only limited validated information on nitrite levels in excipients has been available until now. This has driven the creation of a database to store and share such validated information. The database, maintained by Lhasa Limited, constitutes a central platform to hold the data donated by the pharmaceutical company members on the nitrite concentrations in common excipients measured with validated analytical procedures. As such, 678 data points on 79 excipients, from different lots, and suppliers have been collected in the Nitrites in Excipients Database [1,5].

The article by Hu et al [2] called our attention, since the nitrite measurements reported are considerably higher from those observed in our experience and captured in the database, prompting scrutiny from this group. For example, the nitrite level of 95.6 ppm in sample 7, Ranitidine (drug product), does not compare with any of the levels found in the nitrites in excipients database.

Due to the importance of the topic, we have taken a deeper look at the data and methodology provided and has raised the possibility that analytical artifacts might be at play here, which is expressed in the points below.

2. Discussion

In their article, the authors present results for dimethylamine (DMA) and nitrite from the analysis of seven pharmaceutical samples, i.e., five drug products containing the APIs Diphenhydramine HCl (2x), Ranitidine HCl, Losartan potassium and Metformin HCl, as well as for the undiluted APIs Losartan potassium and Metformin HCl. The undiluted APIs were sourced from a life science company and may therefore not be fully equivalent to pharmaceutical grade API in terms of quality, purity, and impurity profile.

Metformin hydrochloride of pharmaceutical quality is synthesized at commercial scale from dimethylamine (DMA) hydrochloride (HCl) and cyanoguanidine in organic solvent, followed by a solvent exchange to water, filtration and crystallization(s), and finally centrifugation and drying. Nowhere is nitrite added intentionally to the process, and neither organic solvent, DMA HCl nor cyanoguanidine are known to introduce relevant amounts of nitrite. The process water is typically obtained from city water, in which nitrite is commonly restricted to max 1 μg/g and actual values are below 0.1 μg/g. Our own analyses of 36 commercial batches Metformin HCl with Ph.Eur. quality detected 0.01–0.06 μg/g nitrite using the Griess reaction followed by HPLC separation and detection by UV. In comparison, the authors claim to detect 27.0 μg/g and 6.86 μg/g nitrite in one batch Metformin HCl of non-pharmaceutical quality and one batch Metformin tablets, which typically contain ca. 90–95% API of pharmaceutical quality and single digit concentrations of excipients such as povidone and microcrystalline cellulose and max 1% magnesium stearate. Using validated methods, we have measured the following maximum nitrite concentrations in the indicated number of samples from a variety of suppliers (2021.3.0 version of database),

Povidone	52 lots	max 2.3 ppm
Microcrystalline Cellulose (MCC)	73 lots	max 2.4 ppm
Magnesium stearate	44 lots	max 6.1 ppm

Making a worst-case calculation for a typical Metformin tablet formulation the maximum expected nitrite concentration based on our data would be:

Component	Max nitrite (μg/g)	Percentage of formulation	Max nitrite contribution (μg/g tablet)
Metformin HCl	0.06	0.9	0.05
Povidone	2.3	0.045	0.10
MCC	2.4	0.045	0.11
Magnesium stearate	6.1	0.01	0.06
Max total nitrite concentration in the tablet (μg/g tablet)			***0.33***
Max total nitrite concentration in the tablet (μg/g API)			***0.36***

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This value is 20 times lower than the 6.86 μg/g claimed by the authors. Likewise, assuming correctness of our data for povidone, MCC and magnesium stearate, the Metformin API used for producing the tested product batch should have contained 7.32 μg/g nitrite. Considering our data of max 0.06 μg/g nitrite and the design of the API manufacturing process, this number as well as the results claimed by the authors for Metformin HCl, and Metformin tablets of 27.0 μg/g and 6.86 μg/g do not appear plausible but orders of magnitude too high. Naturally, we can't exclude that Metformin and excipients from other sources than the ones we tested may contain higher nitrite concentrations.

We also checked the chemical synthesis pathways for the other APIs included in the study, i.e., Diphenhydramine and Ranitidine and could not identify any potential source of nitrite such as the use of sodium nitrite or reagents likely to be contaminated with nitrite. Losartan is an exception, as nitrite may have been used to quench residual NaN_3 used in the synthesis process. In analogy to our considerations regarding Metformin tablets, we are not aware of any excipients that would contain enough nitrite to cause the high levels the authors claim to have measured in the Losartan, Diphenhydramine and Ranitidine drug products. However, as the authors reported nitrite concentrations relative to the API, the contribution of nitrite from excipients could only be estimated.

Methodology flaws have been raised as possible root-causes for the high values provided, which may constitute analytical artifacts:

a. Sample preparation:
   i. A significant number of syringe filters contain levels of nitrite and therefore could have led to overestimation of the nitrite levels reported during analysis. This was experienced and confirmed by several companies that donated data to the Nitrites in Excipients Database [1,5].
   ii. The manuscript highlights that the samples were sonicated until they were dissolved. Not all products would have been fully dissolved, even after extended sonication as excipients such as magnesium stearate (used in Metformin products) are insoluble in water. It is likely that for this reason the centrifugation and filtration step were included.
   iii. The sonication time is also not specified which causes serious concerns regarding the viability of the procedure. The formation of both nitrite and nitrate upon sonication of aerated water is well-known [3]. Analysis within the consortium highlighted that extended sonication for certain excipients including magnesium stearate generate significant levels of artefactual nitrite during sample preparation.
b. Methodology
   i. It is claimed that the methods are validated however the tests discussed do not provide sufficient data to demonstrate that the methods are fit for purpose, for example:
      - Specificity, the most important validation parameter has not been demonstrated for the analysis of nitrite in the different sample matrices. Data has been collected using both a UV and conductivity detector, representative chromatograms should be provided to demonstrate the method is specific for the analyte. Additionally, there was access to IC-MS technology, which could have been used to demonstrate specificity and peak purity. Specificity might have been demonstrated for common inorganic anions; however, many organic compounds absorb UV light at 210 nm and specificity for common organic impurities for each product has not been demonstrated. Furthermore, the chromatographic method does not seem to be robust as the baseline does not reach the starting height once a run is completed, see Figures 6 and 7 of the original publication.
      - The signal-to-noise ratio has only been calculated in standard solutions and does not provide a true representation in each sample matrix.
      - The precision data reported is based on 3 standard injections (of a high-level standard) over 3 days. This does not provide any representation of the repeatability or precision of the method in the presence of the sample matrix.
   ii. There has been no mention around preparation blanks so cross-contamination of nitrite cannot be ruled out.
   iii. Due to the small sample concentration used, any variability in the method impacts the reported results more significantly in comparison to using larger sample concentrations.
   iv. Analysis was performed on single tablets only; this does not provide any representative data due to tablet variability. Nevertheless, the levels of nitrite reported seem artificially high.
   v. A quadratic fit for the trend line was used for the analysis of DMA. If this was used to quantify nitrite, this could cause inaccuracies at the extremes of the calibration curve.
c. Other points

The authors claim that their method is more sensitive than spectrophotometry and reference a publication from Narayana et al. [4] with an LOD of 930 μg/L. Commercially available test kits for nitrite determination in aqueous solution reach LOQs of down to 30 μg/L, which is about 30-fold lower than reported by the authors of this publication [4]. Therefore, their statement is not valid.

Declarations

Author contribution statement

All authors listed have significantly contributed to the development and the writing of this article.

Declaration of interests statement

The authors declare the following conflict of interest: The authors Joerg Schlingemann, Sebastian Hickert, Giorgio Blom, and Leonardo Allain are employed by companies that manufacture pharmaceuticals subject to regulations regarding maximum nitrosamine limits.

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