APPLICATION NOTE

Quantification of Amyl Nitrites in Inhalant Mixtures Using Quantitative NMR

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qNMR

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Introduction

Inhalants come in many different forms, which can be classified according to their chemical structures: aliphatic hydrocarbons, aromatic hydrocarbons, ketones, haloalkanes, nitrites, and nitrous oxide.[1] While many inhalants have important medical uses, these have also commonly been used recreationally to induce intoxication or produce psychoactive reactions.[2] As a result, these products are commonly regulated, and the formulations designated for medical use must be thoroughly tested to ensure quality and safety. Nitrite-based inhalants are commonly used for their vasodilating effects and can be tested according to the *USP31-NF26* testing method.[3] These types of products contain mixtures of 3-methylbutyl nitrite (isoamyl nitrite) and 2-methylbutyl nitrite, which are the nitrite esters of 3-methyl-1-butanol and 2-methyl-1-butanol, respectively. Additionally, these inhalants will include a suitable stabilizer, such as linseed oil epoxy resin.

Due to commercial availability, and to analyze molecules that are representative of amyl nitrite inhalants, mixtures of isoamyl nitrite, 2-methyl-1-butanol, and 3-methyl-1-butanol were prepared. Because 2-methyl-1-butanol and 2-methylbutyl nitrile are structurally similar, and the molecular weights of isoamyl nitrite and 2-methylbutyl nitrite are the same, the method presented in this study would be identical for the analysis of real inhalant samples. A summary of common amyl nitrite inhalant mixtures and those analyzed in this study are presented in **Figure 1**.

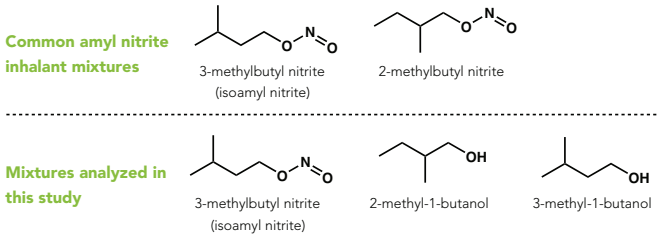

Figure 1. *Structures of common amyl nitrite inhalant mixtures (top) and mixtures analyzed in this study (bottom).*

The *USP-31-NF26* testing method requires that these products contain at least 80.0% and no more than 105.0% of amyl nitrite.[3] The amyl nitrite content is accurately determined using quantitative NMR (qNMR), with benzyl benzoate as an internal calibrant. While some of the signals in the ^{1}H NMR spectra of these mixtures do overlap, there are unique and well-separated signals that can be used for quantification. Specifically, the protons highlighted in **Figure 2** can be used for this purpose.

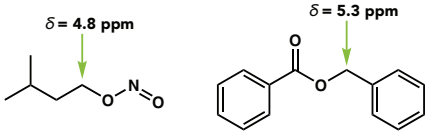

Figure 2. *Protons used for quantification in this study indicated by green arrows, with respective chemical shifts included. Left: isoamyl nitrite; right: benzyl benzoate.*

The ^{1}H NMR spectra for each compound relevant to this study were acquired in chloroform-*d* to confirm their respective chemical shifts. A stacked plot of these spectra is presented in **Figure 3**. The use of qNMR necessitates a few key considerations to ensure success.[4] Namely, the signals from the internal calibrant must not overlap with those from the analytes of interest, the calibrant must be stable in the medium of analysis, and it must not react with other species in solution. Additionally, a sufficient interscan delay must be chosen to allow full relaxation of the spins of interest between scans. This is typically done be selecting a delay corresponding to 5 times the value of the longest T_1. Finally, accurate weighing using an analytical balance and proper liquid dispensing techniques are necessary to ensure optimal results.

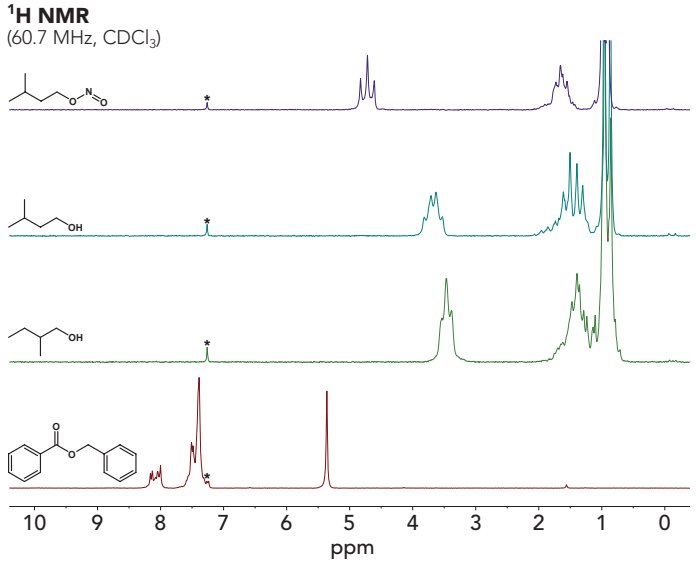

Figure 3. *Stacked ^{1}H (60.7 MHz) NMR spectra of benzyl benzoate (internal calibrant), 2-methyl-1-butanol, 3-methyl-1-butanol, and isoamyl nitrite. The asterisks represent the residual solvent peaks for chloroform-d.*

In this work, 3 samples corresponding to approximately 75 wt%, 85 wt%, and 95 wt% of isoamyl nitrite were prepared and analyzed using ^{1}H (60 MHz) benchtop NMR. These mixtures are representative of the typical amyl nitrite ranges requires in these types of products according to the *USP31-NF26* testing method. The isoamyl nitrite content was quickly quantified using qNMR, demonstrating that this approach is accurate and straightforward for this application.

The samples were prepared by accurately weighing isoamyl nitrite, 2-methyl-1-butanol, 3-methyl-1-butanol, and benzyl benzoate (internal calibrant) in the same vial. The mixtures were dissolved in chloroform-*d* and transferred to NMR tubes. The samples were allowed to stabilize for 10 minutes in the instrument and the T_1 values for the signals presented in **Figure 2** were determined for each sample. Then, spectra were acquired in triplicate for each sample to confirm reproducibility and accuracy. The following equation was used to calculate the isoamyl nitrite wt% in each sample:

$$\textit{Isoamyl Nitrite (wt\%)} = \frac{I_{isoamyl}}{N_{isoamyl}} \cdot \frac{N_{IC}}{I_{IC}} \cdot \frac{m_{IC}}{MW_{IC}} \cdot \frac{MW_{isoamyl}}{m_{sample}} \cdot P_{IC} \cdot 100$$

Where: ***isoamyl*** = isoamyl nitrite; ***IC*** = internal calibrant (benzyl benzoate); ***I*** = integration area; ***N*** = number of protons associated with the integrated signals; ***m*** = mass obtained from analytical balance; ***MW*** = molecular weight; m_{sample} = combined masses of isoamyl nitrite, 2-methyl-1-butanol, and 3-methyl-1-butanol; ***P*** = purity.

A spectrum of the 75 wt% mixture with the respective integration areas for the benzyl benzoate and isoamyl nitrite signals are shown in **Figure 4**. Additionally, the qNMR results for all samples are summarized in **Table 1**. In all spectra, the chemical shifts were referenced using the benzyl benzoate signal at 5.30 ppm.

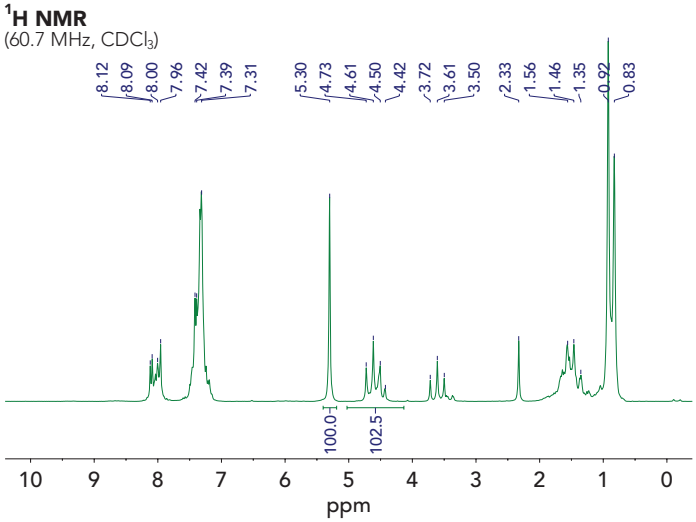

Figure 4. *^{1}H (60.7 MHz) NMR spectrum of 75 wt% mixture of isoamyl nitrite with 2-methyl-1-butanol, 3-methyl-1-butanol, and benzyl benzoate as the internal calibrant. The integration areas for benzyl benzoate (normalized to 100) and isoamyl nitrite are shown.*

The qNMR values obtained from these experiments match the actual mixture compositions very closely, as determined from the analytical balance values. With the required scan delays, each spectrum took approximately 5 minutes to acquire and the relative standard deviation (RSD) values between runs were very low, confirming that this approach can be used to successfully quantify amyl nitrites in inhalant mixtures using qNMR on a benchtop instrument. By focusing on signals not suffering from overlap with other chemical species, the entire molecule can be quantified, which is an important benefit of qNMR.

Table 1. Summary of qNMR results for the quantification of isoamyl nitrite in different mixtures.

	Isoamyl Nitrite (wt%)		
Actual Composition	**73.6**	**85.3**	**95.3**
Run 1	**71.7**	**83.5**	**94.5**
Run 2	**71.6**	**83.4**	**94.5**
Run 3	**72.0**	**83.4**	**94.4**
Average*	**71.8 (0.2)**	**83.4 (0.1)**	**94.5 (0.1)**

*Relative standard deviation values are shown in parentheses

Conclusion

The work performed herein demonstrates the ease with which benchtop NMR can be used to quantify amyl nitrites in inhalants, using benzyl benzoate as an internal calibrant for qNMR. Once the appropriate acquisition parameters have been determined, these analyses could easily be streamlined. Additionally, the mixtures analyzed in this study are representative of the typical ranges observed in these types of product compositions.

References

(1) Brouette, T.; Anton, R. *Am. J. Addict.* **2001**, *10*, 79–94.

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(3) USP Monographs: Amyl Nitrite Inhalant (*USP31-NF26*): http://www.uspbpep.com/usp31/v31261/usp31nf26s1_m4600.asp (accessed Nov 23, 2021).

(4) Rundlöf, T.; Mathiasson, M.; Bekiroglu, S.; Hakkarainen, B.; Bowden, T.; Arvidsson, T. *J. Pharm. Biomed. Anal.* **2010**, *52*, 645–651.

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