APPLICATION NOTE

Quantification of Amyl Nitrites in Inhalant Mixtures Using Quantitative NMR
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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. While many inhalants have important medical uses, these have also commonly been used recreationally to induce intoxication or produce psychoactive reactions. As a result, these products are have also commonly been used recreationally to induce intoxication and can be tested according to the USP31-NF26 based inhalants are commonly used for their vasodilating effects use must be thoroughly tested to ensure quality and safety. Nitrite-commonly regulated, and the formulations designated for medical study are presented in Figure 1.

Due to commercial availability, and to analyze molecules that are representative of amyl nitrite inhalants, mixtures of isoamyl nitrite, 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.

In this work, 3 samples corresponding to approximately 75 wt%, 85 wt%, and 95 wt% of isoamyl nitrite were prepared and analyzed using 1H (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- and transferred to NMR tubes. The samples were allowed to stabilize for 10 minutes in the instrument and the T1 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:

\[
\text{Isoamyl Nitrite (wt%)} = \frac{I_{\text{isoamyl}}}{N_{\text{isoamyl}}} \cdot \frac{m_{\text{isoamyl}}}{MW_{\text{isoamyl}}} \cdot \frac{100}{m_{\text{sample}}} = \text{Purity}
\]

Where: \( \text{isoamyl} = \) isoamyl nitrite; \( I_{\text{C}} = \) 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; \( m_{\text{sample}} = \) 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.

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 nitrates 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.

<table>
<thead>
<tr>
<th>Actual Composition</th>
<th>Isoamyl Nitrite (wt%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>73.6</td>
</tr>
<tr>
<td>Run 1</td>
<td>71.7</td>
</tr>
<tr>
<td>Run 2</td>
<td>71.6</td>
</tr>
<tr>
<td>Run 3</td>
<td>72.0</td>
</tr>
<tr>
<td>Average*</td>
<td>71.8 (0.2)</td>
</tr>
</tbody>
</table>

*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 nitrates 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