Leading Performance

Chromatographic Based Approaches for the Identification and Quantitation of Adulterants Present in Glutaraldehyde Products

Overview

Glutaraldehyde is a versatile microbiocide that has shown utility in numerous applications for controlling microbes found in industrial operations such as oilfields, cooling towers and high-level disinfection of medical equipment.

During the past few years, some formulators have been engaged in deliberately blending genuine glutaraldehyde with other aldehydes, namely formaldehyde and glyoxal, with the overall objective of making financial gains. Unfortunately, these adulterated glutaraldehyde mixtures cannot be distinguished from genuine glutaraldehyde using standard aldehyde-based titration methods. These deceptive acts can result in customers overpaying by 50 to 200 percent based on current average market prices.

We present a number of derivatization-based chromatographic approaches to identify and quantitatively determine the presence of formaldehyde and glyoxal in glutaraldehyde-based products. The use of the 2, 4 dinitrophenylhydrazine derivatization procedure followed by gradient elution reverse-phase chromatography resulted in the complete separation and unambiguous identification of glutaraldehyde and formaldehyde in a single analysis. 3-methyl-2-benzothiazolinone (MBTH) derivatization and an isocratic elution reverse-phase chromatography resulted in the selective identification of glyoxal in a glutaraldehyde-based product. These methods are capable of detecting down to 1% of formaldehyde and glyoxal, respectively, and provide customers with powerful analytical tools for distinguishing genuine glutaraldehyde from adulterated products.

Introduction

Dow Glutaraldehyde is:

- Formaldehyde free
- A versatile microbiocide with broad efficacy
- Readily biodegradable
- Non-carcinogenic
- Non-persistent
- Non-bio-accumulative

\[
\text{CH}_2\text{CH}(\text{CH}_2\text{CO})\text{H}
\]
Analytical Issues

- Glut concentration is typically determined by titration based on carbonyl reaction with hydroxylamine to release HCl.
- This method is not glutaraldehyde specific, as other aldehydes react in a similar scheme, resulting in potential bias.
- Direct detection of glut by UV is difficult due to absence of a chromophore.
- Derivatization reagents such as 2,4 dinitrophenyl hydrazine (DNPH), have been used for detection of aldehydes.
- Glyoxal presents interesting challenges with DNPH, as the derivative formed is insoluble in ACN and also co-elutes with the glut-DNPH derivative.
- Another derivatization reagent useful for the determination of aldehydes is 3-methyl-2-benzothiazolinone hydrazone (MBTH) was determined to be ideal for the detection of glyoxal.

Schematic of issues with titration

Experimental Method 1

Detection of formaldehyde (FA) in glutaraldehyde (GA) products

Pre-column derivatization by 2,4dinitrophenylhydrazine

\[
\begin{align*}
\text{2,4-dinitrophenylhydrazine} & \quad \text{Aldehyde/ketone} \\
\text{Hydrazone derivative} & \quad \text{Results 50\% GA} \\
\text{Results 50\% GA} & \quad \text{Chromatography}
\end{align*}
\]
Sample Preparation
A glutaraldehyde and formaldehyde sample was prepared by dissolving an aliquot of sample in acetonitrile in a 1:5000 dilution, resulting in an expected final concentration of approximately 100 ppm glutaraldehyde in the sample.

Preparation of 2,4-Dinitrophenylhydrazine (2,4-DNPH)
1. 2,4-DNPH was prepared by dissolving 0.5g DNPH in 98.5 mL of acetonitrile followed by acidification with phosphoric acid (1.5 mL of 85% H₃PO₄).

Derivatization Procedure
1. 0.8 mL of DNPH solution was added to 2 mL of sample/standard using a pipette.
2. Sample was allowed to incubate for an hour at room temperature before analysis.

**Figure 1**

(A) Representative chromatogram of 100 ppm of Dow genuine glutaraldehyde showing glutaraldehyde peak at a retention time of ~18 minutes.

(B) Representative chromatogram of a “competitor” sample containing both glutaraldehyde and formaldehyde seen at retention time ~15 minutes. Formaldehyde peak was confirmed by comparing retention times to a formaldehyde standard.

**Gradient Conditions:**
- Time-min: 0, 3, 8, 12, 14, 18, 21, 25, 26, 30
- A-water: 95, 90, 85, 75, 50, 30, 30, 90, 95
- B-ACN: 5, 10, 15, 25, 50, 70, 70, 10, 5

**Other Details:**
- Agilent 1100 HPLC
- λ = 365 nm
- Flow rate: 2.5 mL/min
- Injection vol = 20μL
- Column: Sphericlon ODS1 5 micron, 4.6mm x 150mm
Figure 2

A

Glutaraldehyde Data – Standard range: 1 to 200 ppm

\[
y = 35.93x - 0.0288 \\
R^2 = 1
\]

B

Formaldehyde 1 to 50 ppm

\[
y = 54.402x - 5.1108 \\
R^2 = 1
\]

Figure 2 (A) Linear least squares regression plot of several standards of glutaraldehyde ranging from 1 ppm-50 ppm with an \( R^2 \) of 1. (B) Linear least squares regression plot of standards of formaldehyde ranging from 1 ppm to 200 ppm with an \( R^2 \) value of 1. All data points are an average of duplicate injections.

Figure 3

A

<table>
<thead>
<tr>
<th>Sample</th>
<th>% GA Experimental Results</th>
<th>% FA Experimental Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>29.94</td>
<td>9.94</td>
</tr>
<tr>
<td>2</td>
<td>29.92</td>
<td>9.99</td>
</tr>
<tr>
<td>3</td>
<td>29.69</td>
<td>9.98</td>
</tr>
<tr>
<td>4</td>
<td>29.75</td>
<td>10.02</td>
</tr>
<tr>
<td>5</td>
<td>29.56</td>
<td>9.94</td>
</tr>
<tr>
<td>6</td>
<td>29.41</td>
<td>9.95</td>
</tr>
<tr>
<td>N=6</td>
<td>Average = 29.71</td>
<td>Average = 9.97</td>
</tr>
<tr>
<td></td>
<td>Standard Deviation = 0.20</td>
<td>Standard Deviation = 0.03</td>
</tr>
<tr>
<td></td>
<td>RSD = 0.69</td>
<td>RSD = 0.30</td>
</tr>
</tbody>
</table>

Figure 3: (A) Table showing precision data for the HPLC method for formaldehyde in glutaraldehyde obtained by analyzing a mock sample containing 30% GA and 10% FA six times.
<table>
<thead>
<tr>
<th>Analyte (Sample)</th>
<th>Number of samples</th>
<th>Concentration Range (ppm)</th>
<th>Average Recovery (%)</th>
<th>Range of Recoveries (%)</th>
<th>Standard Deviation of Recoveries (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutaraldehyde</td>
<td>6</td>
<td>29.97–39.99</td>
<td>96.84</td>
<td>95.37–97.63</td>
<td>0.62</td>
</tr>
<tr>
<td>Formaldehyde</td>
<td>6</td>
<td>1.01–10.31</td>
<td>101.04</td>
<td>99.80–102.90</td>
<td>1.10</td>
</tr>
</tbody>
</table>

Figure 3: (B) Accuracy data from the analysis of several samples containing known levels of glutaraldehyde and formaldehyde.

### Figure 4

<table>
<thead>
<tr>
<th>Samples</th>
<th>Glutaraldehyde Conc. by HPLC (%)</th>
<th>Formaldehyde Conc. by HPLC (%)</th>
<th>Glutaraldehyde Conc. by titration (%)</th>
<th>Spec-GA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Competitor 1</td>
<td>29.23</td>
<td>13.61</td>
<td>49.54</td>
<td>50.0–51.5</td>
</tr>
<tr>
<td>Competitor 2</td>
<td>50.51</td>
<td>0</td>
<td>49.66</td>
<td>50.0–51.5</td>
</tr>
<tr>
<td>Competitor 3</td>
<td>20.82</td>
<td>18.04</td>
<td>48.72</td>
<td>50.0–51.5</td>
</tr>
<tr>
<td>Competitor 4</td>
<td>49.17</td>
<td>0</td>
<td>49.73</td>
<td>50.0–51.5</td>
</tr>
<tr>
<td>Dow Glut Lot: ZA1955S4D1</td>
<td>49.21</td>
<td>0</td>
<td>50.42</td>
<td>50.0–51.5</td>
</tr>
</tbody>
</table>

Figure 4: Table displaying the values of glutaraldehyde determined for several competitor samples and genuine Dow product by HPLC-DNPH derivatization method and by titration method. Results by titration show samples to be near expected 50% concentration. However, this method does not differentiate between the different aldehydes.

### Experimental Method 2

**Detection of glyoxal in glutaraldehyde products**

\[
\begin{array}{c}
\text{Glutaraldehyde} \\
\text{Glyoxal}
\end{array}
\]

MBTH reagent selectively reacts with glyoxal, forming a yellowish diazine species that can be detected by UV.

**Pre-column derivatization by 3-methyl-2-benzothiazolinone hydrazone (MBTH)**

\[
\begin{array}{c}
\text{MBTH} \\
\text{Glyoxal} \\
\text{MBTH-Glyoxal diazine derivative} \\
\text{Water}
\end{array}
\]

**Sample Preparation**

A sample containing glyoxal and formaldehyde or glutaraldehyde by dissolving an aliquot of the sample in water at 1:30000 dilution.

**Preparation of 3-methyl-2-benzothiazolinone hydrazone (MBTH)**
1. 3-methyl-2-benzothiazolinone hydrazone (MBTH) was prepared by dissolving 1.0g MBTH in 60% by weight of glacial acetic acid and stirring gently until all the MBTH was dissolved.

Derivatization Procedure
1. Take 2 mL of sample/standard and add 0.8 mL of MBTH solution using a pipette into a 10 ounce vial.
2. Sample was allowed to incubate for an hour at 70ºC before analysis by HPLC after filtration

HPLC Results (Glyoxal)

Figure 5

(A) Chromatogram of 3 ppm of a glyoxal standard derivatized by MBTH method.
(B) Chromatogram of 3 ppm glyoxal prepared from a sample containing 15% glutaraldehyde, 15% glyoxal and 5% formaldehyde.

Figure 6

Linear least squares regression plot of several standards of glyoxal ranging from 1 ppm-10 ppm with an $R^2$ of 1
Figure 7

A

<table>
<thead>
<tr>
<th>Trial #</th>
<th>Mock A 20% Gly/10% FA</th>
<th>Mock B 10% Gly/30% GA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20.62</td>
<td>11.43</td>
</tr>
<tr>
<td>2</td>
<td>20.25</td>
<td>10.35</td>
</tr>
<tr>
<td>3</td>
<td>19.64</td>
<td>10.56</td>
</tr>
<tr>
<td>4</td>
<td>20.06</td>
<td>10.79</td>
</tr>
<tr>
<td>5</td>
<td>20.95</td>
<td>11.04</td>
</tr>
<tr>
<td>N=5</td>
<td>Average = 20.30</td>
<td>Average = 10.83</td>
</tr>
<tr>
<td></td>
<td>Standard Deviation = 0.51</td>
<td>Standard Deviation = 0.42</td>
</tr>
<tr>
<td></td>
<td>RSD = 2.50</td>
<td>RSD = 3.89</td>
</tr>
</tbody>
</table>

Figure 7 (A): Table showing precision data for the HPLC method for glyoxal obtained by analyzing a “mock” sample containing 30% GA and 10% glyoxal five times. (B) Accuracy data from the analysis of several samples containing known levels of glyoxal.

B

<table>
<thead>
<tr>
<th>Analyte (Sample)</th>
<th>Number of Samples</th>
<th>Concentration Range (ppm)</th>
<th>Average Recovery (%)</th>
<th>Range of Recoveries (%)</th>
<th>Standard Deviation of Recoveries (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glyoxal</td>
<td>6</td>
<td>1–10</td>
<td>98.67</td>
<td>93.10–104.82</td>
<td>5.1</td>
</tr>
</tbody>
</table>

Discussions and Conclusions

- The use of 2,4 dinitrophenylhydrazine followed by an HPLC separation is ideal for formaldehyde detection in the presence of glutaraldehyde.
- DNPH reagent concentration dependency can limit the number of aldehyde species detected.
- Glyoxal-DNPH derivative was found to be insoluble in organic solvent systems, thus making it unsuitable for detection of glyoxal in glut products.
- 3-methyl-2-benzothiazolinone hydrazone selectively reacts with glyoxal in the presence of other aldehydes, resulting in a diazine species that is detected by LC-UV approach.
- Glutaraldehyde products from Dow do not contain glyoxal or formaldehyde.

References


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