A Fast and Sensitive Method for Residual Hydrazine Analysis in Pharmaceutical Samples

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Hydrazine Properties

H₂N—NH₂

Hydrazine is

- a highly reactive molecule widely used in chemical syntheses of intermediates and active pharmaceutical ingredients
- a known carcinogen - can have an impact on product risk assessment if present in the final drug substance and drug product as an impurity.
- a small, polar, basic molecule with no chromophore
Objective

Develop a method for detection of low (ppm) level of hydrazine in pharmaceutical samples and wash solutions. Method criteria:

- simple sample preparation step
- efficiency
- sensitivity
- ease of transfer
Control of Genotoxic Impurities in Pharmaceuticals

According to current regulatory guidance, it is assumed that genotoxic compounds have the potential to damage DNA at very low level of exposure. Thus, actual and potential impurities most likely to arise during synthesis, purification, and storage should be identified. Both the EMEA guidelines and a PhRMA white paper propose a limit of 1.5 µg/day for genotoxic impurities in pharmaceuticals.
Analytical Challenges in Setting Limits for Genotoxic Impurities

- Low level (ppm) needs to be detected
- Many genotoxic impurities are highly reactive and therefore difficult to analyze
- Sample matrix can be chemically similar and present at extremely high level
- Techniques being employed aren’t standard and may present significant technology transfer issues
Existing methods for hydrazine

- are mostly applicable to environmental samples (water, air, soil)
- employ sophisticated equipment and/or derivatization
- tend to be cumbersome and time consuming
- are subject to interference, especially by hydrazine derivatives (colorimetric and titrimetric methods)
Advantages of using Acetone:
- Serves as both diluent and derivatizing agent
- Symmetrical molecule (produces only one isomer)
- Fast reaction rate
- Low toxicity
- Good GC separation from the product of the reaction.
GC Analysis of Acetone Azine

Oven Program:
Initial temperature: 95°C

<table>
<thead>
<tr>
<th></th>
<th>Rate</th>
<th>Final</th>
<th>Hold Time</th>
<th>Total Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10.0</td>
<td>120.0</td>
<td>0.00</td>
<td>3.50</td>
</tr>
<tr>
<td>2</td>
<td>30.0</td>
<td>225.0</td>
<td>4.00</td>
<td>11.00</td>
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<tr>
<td>3</td>
<td>40.0</td>
<td>95.0</td>
<td>1.00</td>
<td>15.25</td>
</tr>
</tbody>
</table>

Column: 20-m length x 0.18-mm i.d., 1μm film thickness, 6% cyanopropylphenyl-94% dimethylpolysiloxane (DB-624);
Detector: FID, 280°C; Injector: 1 μL into the split port maintained at 200 °C;
Carrier Gas: Helium; Column Flow: 1.3 ml/min; Split ratio/Split Flow: 110/143 ml/min.
Linearity Data for Acetone Azine

\[ y = 105.48x + 0.0297 \]
\[ R^2 = 0.9999 \]

<table>
<thead>
<tr>
<th>Concentration</th>
<th>mg/mL</th>
<th>ppm</th>
<th>Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.000842</td>
<td>0.84</td>
<td>0.1168</td>
<td></td>
</tr>
<tr>
<td>0.005052</td>
<td>5.05</td>
<td>0.5077</td>
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<tr>
<td>0.00842</td>
<td>8.40</td>
<td>0.8384</td>
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</tr>
<tr>
<td>0.05052</td>
<td>50.5</td>
<td>5.2658</td>
<td></td>
</tr>
<tr>
<td>0.0842</td>
<td>84.2</td>
<td>8.8097</td>
<td></td>
</tr>
<tr>
<td>0.5052</td>
<td>505.2</td>
<td>54.1033</td>
<td></td>
</tr>
<tr>
<td>0.842</td>
<td>842.0</td>
<td>88.3914</td>
<td></td>
</tr>
</tbody>
</table>

S/N at 0.84 ppm is \(~10:1\)
0.84 ppm of Acetone azine corresponds to 0.24 ppm of free hydrazine in solution or 2.4 ppm/100 mg of sample
Conversion of Hydrazine Mono-Hydrate to Acetone Azine: GC/MS Data

\[ \text{H}_2\text{N} - \text{NH}_2 + 2 \text{O} - \text{CH}_3 \rightarrow \text{H}_3\text{C} - \text{N} - \text{NH}_2 \rightarrow \text{H}_3\text{C} - \text{N} \equiv \text{CH}_3 \]

MW = 72

MW = 112

\[ \text{acetone} - 1.007 \]

\[ \text{mono-substituted} - 2.010 \]

\[ \text{Acetone azine} - 2.885 \]

\[ \text{pA} \]

Minutes

Bristol-Myers Squibb Company
Conversion Rate of Hydrazine Mono-Hydrate to Acetone Azine

<table>
<thead>
<tr>
<th>Time, min</th>
<th>% Conversion</th>
</tr>
</thead>
<tbody>
<tr>
<td>103</td>
<td>22.8</td>
</tr>
<tr>
<td>153</td>
<td>29.1</td>
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<tr>
<td>170</td>
<td>31.7</td>
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<tr>
<td>187</td>
<td>34.1</td>
</tr>
<tr>
<td>204</td>
<td>36.8</td>
</tr>
<tr>
<td>259</td>
<td>37.5</td>
</tr>
<tr>
<td>276</td>
<td>39.7</td>
</tr>
<tr>
<td>573</td>
<td>69.1</td>
</tr>
<tr>
<td>590</td>
<td>77.0</td>
</tr>
</tbody>
</table>
Reaction Conversion of Hydrazine Mono-Hydrate to Acetone Azine Catalyzed by Acid

<table>
<thead>
<tr>
<th>Time, min</th>
<th>% Conversion, Acetone Hydrazone/ Acetone Azine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HCOOH</td>
</tr>
<tr>
<td>0</td>
<td>0/100</td>
</tr>
<tr>
<td>15</td>
<td>0/100</td>
</tr>
<tr>
<td></td>
<td>CH3COOH</td>
</tr>
<tr>
<td></td>
<td>5.5/94.5</td>
</tr>
<tr>
<td></td>
<td>0/100</td>
</tr>
</tbody>
</table>

Conclusion: The derivatization proceeds in less than 3 min in the presence of formic acid and within ~15 min with Acetic Acid.
Reaction Conversion of Hydrazine Dihydrochloride to Acetone Azine

- **Problem:**
  Low solubility of the salt in acetone
  Conversion rate is ~50% within 24 hours

- **Solution:**
  Standard prep
  - dissolved~ 10 mg/2 ml of H2O
  - diluted with acetone to 200 ml
  The derivatization proceeds in 2.5 hours (97.5%). Presence of formic acid did not show improvement in the reaction rate
Sample Analysis for Residual Hydrazine.

Problem:
~100 mg needed to be dissolved in 1 ml of acetone for ppm level hydrazine analysis.

Solution:
Sample prep
- added 1 ml of 1/99 H2O/Acetone to 100 mg of the sample.
- Heated at~ 40°C. Mixed well.
The solution became clear for a short period of time (1-2 minutes) and then the precipitate formed again under cooling to room temperature.

Question:
Did the reaction occur or did the recrystallization take place?
Compound A

Precipitate from Acetone, yield is \( \sim \) 100%

DMSO-d6, 400 MHz
$^1$H NMR Spectra Comparison (2)

DMSO-d$_6$, 400 MHz

Acetone layer

Precipitate from Acetone spiked with Acetone Azine (AA)
NMR Study of reaction conversion

- NMR of the precipitate showed mostly hydrazone, the conversion is close to 100% by weight.
- NMR of supernatant showed ~30% of unreacted sample, hydrazone and acetone azine formed due to the presence of residual hydrazine in the sample.
- Spiking experiment showed 100.9% recovery of spiked hydrazine (synergistic effect?)
Residual Hydrazine Analysis in Neutral Molecules with Low Solubility in Acetone

Procedure:
- Dissolved sample (~50mg) in NMP or any other suitable solvent
- Added 0.1 ml of acetone/HCOOH;
- Analyzed sample by the method described above

The recovery of hydrazine from solution fortified with hydrazine mono-hydrate was 103%
Direct Analysis of Hydrazine in Aqueous Solutions

Mixed-Mode Chromatography:

Column: Primesep 100 (Sielc Technology)
Gradient: 20%ACN/80%H2O/0.2%TFA for 6 min, then 80%ACN/20%H2O/0.2%TFA, 1 ml/min, Sample concentration - 31.4 mg/ml, inj. Vol.- 10 µl
Detection: ELSD; T= 40°C; Photomultiplier voltage- 500
Direct Analysis of Hydrazine by Mixed-Mode Chromatography

**ELSD**

Calibration Curve for Hydrazine Dihydrochloride

\[ y = 159952x^{1.3903} \]

\[ R^2 = 0.9992 \]

Detection limit - ~100 ppm

**CAD**

Calibration Curve for Hydrazine Monohydrate

\[ y = 892671x - 6081.7 \]

\[ R^2 = 0.9972 \]

Detection limit - ~35 ppm
Conclusion

- The reaction of acetone with hydrazine free base and HCl salts has been investigated and conditions were developed for quantitative and fast conversion to acetone azine.
- A fast and sensitive GC method for residual hydrazine analysis in pharmaceutical samples has been developed.
- Excellent linear relationship between acetone azine concentration and FID response was demonstrated with minimal detectable concentration 0.24 ppm.
- The method is applicable to pharmaceutical compounds at all stages of development with some modifications.
- Hydrazine can be analyzed in aqueous solutions using mixed-mode chromatography and CAD detection with minimal detectable concentration 35 ppm.
Acknowledgments

- Su Pan (CAD data)
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