**ABSTRACT**

Hydrazine is a human carcinogen present in tobacco and cigarette smoke. A simple method is developed for the qualitative and quantitative determination of hydrazine in tobacco smoke by HPLC with PDA (Photo Diode Array) detector. Cigarette smoke is trapped into an impinger containing a mixture of methanol and 0.1 N sulphuric acid in the ratio (70:30). The smoke extract is derivatized using benzaldehyde solution, where in the hydrazine is converted to benzalazine and subjected for chromatographic separation and monitored by PDA detector at 313 nm. The method has been validated using standard validation protocols, minimum recovery of 76.9% was obtained with a linear regression coefficient of 0.9998 for the range of 20 to 4000 ppb and limit of detection was 12 ppb. The titled method provides several advantages such as fast and easy sample preparation. The method is found suitable for rapid determination of Hydrazine in cigarette smoke.

**INTRODUCTION**

Hydrazine is a volatile colorless liquid, molecular formula H₂NNH₂. It is a severe skin and mucous membrane irritant in humans and in animals, it is a convulsant, carcinogenic and genotoxic. It is highly reactive reducing agent which decomposes slowly in air. It is used in the synthesis of maleic hydrazide.

The GC-MS method reported in literature is for Hydrazine in smokeless tobacco products is tedious and involves time consuming sample preparation steps, the method discussed is used for the determination of Hydrazine in cigarette smoke using HPLC with a Photo diode Array detector.

A few analytical methods:


b) Health and safety Executive General COSHH ACP (Control of substances hazardous to health) and Carcinogens.

Limitation : It is for non-tobacco samples

**SAMPLE PREPARATION**

Cigarette smoking (20 cig) using Rotary smoking machine

Collect the smoke particulate matter on a Cambridge filter pad (CFP) and smoke in an impinger containing 30ml mixture of methanol and 0.1 N Sulphuric acid (70:30)

Transfer (CFP) into a 125 ml Erlenmeyer conical flask to which impinger solution was added. The flask was agitated for 60 min on orbital shaker at 180rpm

Derivatize the smoke and standard solution and inject the derivatized standard and sample to LC system

**STANDARD AND SAMPLE CHROMATOGRAM**

**LINEARITY GRAPH**

The linearity of the method was checked by employing standard solutions of concentration from 20 ppb to 4000 ppb and respective responses were measured and plotted against Hydrazine concentration.

**HPLC-PDA CONDITIONS**

- Column temperature : 30°C
- Injection volume : 20μl
- Isocratic elution :
  - Flow rate : 1.0 ml/min
  - Run time : 12.0 min.
- Column : LichroCART ® 250-4, RP-18 e (4.6 mm x 250mm x 5 um)
- Lambda max : 313 nm
- Benzaldehyde peak at 3.0 min ± 0.5 min.
- Hydrazine peak at 7.3 min ± 0.5 min

**LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION**

Determination of signal-to-noise ratio is performed by comparing measured signals from samples with known low concentration of anlyte with those of blank samples and establishing the minimum concentration at which the analyte can be detected.

1) Signal to noise ratio of 3 : 1 is considered limit of detection.
2) Signal to noise ration of 10 : 1 is considered limit of quantification

**RECOVERY**

Laboratory Fortified samples (LFS) are used to determine the recovery.

Studies conducted by adding a known amount of Hydrazine to the tobacco smoke sample of known concentration.

<table>
<thead>
<tr>
<th>Hydrazine in ppb</th>
<th>Spiked</th>
<th>Recovered</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>40.0</td>
<td>36.8</td>
<td>92.0</td>
</tr>
<tr>
<td>2</td>
<td>80.0</td>
<td>65.7</td>
<td>82.1</td>
</tr>
<tr>
<td>3</td>
<td>160.0</td>
<td>146.3</td>
<td>91.4</td>
</tr>
</tbody>
</table>

Recovery at different range of concentration 40 to 160 ppb is 77 - 91%

**REPEATABILITY AND REPRODUCIBILITY**

Cigarette samples were analyzed for Repeatability and Reproducibility studies. Rsrd was within the limit

**CONCLUSION**

Easy sample preparation, fast analysis and increased productivity and Good resolution of Hydrazine from tobacco impurities