CHAPTER THREE

An Extractive Spectrophotometric method For the Determination of Pyridine in Air and Environmental Samples.
An Extractive Spectrophotometric method
For the Determination of Pyridine in
Air and Environmental Samples.

SUMMARY

A new sensitive spectrophotometric method for determination of pyridine at ppm level is described. The method is based on the reaction of pyridine with cyanogen bromide to form, glutarconic aldehyde, which is subsequently coupled with 4-amino salicylic acid. The yellow polymethine dye formed which is extractable in iso-amyl alcohol, shows an absorbance maxima at 400 nm. The reaction is very sensitive and follows Beer's Law in the range of 0.025 to 0.2 ppm in the extractive system. The method has been successfully applied for the determination of pyridine in waste water, benzene, laboratory air and environmental samples.

INTRODUCTION

Pyridine is a widely used organic solvent in many industrial processes. It is a colourless basic liquid with a characteristic unpleasant odour. It is mostly derived from crude coal tar and can also be synthesized from aliphatic compounds. It was isolated first from bone oil. Pyridine and similar compounds are also present in alkaloids like nicotine, coniine etc. Pyridine is used as a solvent in many chemical processes and also for denaturing ethyl alcohol. It finds use in dye industry, in manufacture of some explosives, rubber, pharmaceuticals, textiles, resins, paints, chemicals and bactericides etc. Pyridine is discharged as waste into the water from coking operation during petroleum processing. Pyridine and similar compounds are stable [1-11].

Pyridine has narcotic action when administered. Effective doses by any route produces weakness, ataxia, unconsciousness and salivation. It is generally absorbed through skin and from gastrointestinal tract and also by inhalation. Transient symptoms of nausea, insomnia, nervousness and low back or abdominal discomfort with urinary frequency have also been observed. After absorption, pyridine is excreted in urine unchanged with a smaller portion methylated. Exposure to pyridine vapours often causes symptoms of moderate mucous membrane irritation. Pyridine affects the central nervous system causing fatigue and dizziness. Diseases like insomnia, kidney injury nervousness and abdominal discomfort is observed due to repeated exposure to pyridine vapours. [1, 11-14].

Pyridine has a very disagreeable odour. The odour threshold limit value for pyridine is less than 1 ppm. The threshold limit value for pyridine has been reduced to 5 ppm by OSHA [15] from 10 ppm, proposed by ACGIH [16].

Several methods have been reported for the determination of pyridine, various techniques employed for the determination are titrimetry including conductometric titration [17], coulometric titration [18,19], and potentiometric titration [20], high frequency conductometric titration [21], differential anodic stripping voltammetry [22], chromatographic methods including gas chromatography [23-27], paper chromatography [28], gas-liquid chromatography [29], capillary gas chromatography [30,31], liquid chromatography [32] etc. Reversible optical wave guide vapour sensor for detection of pyridine has also been developed [33].

Spectrophotometric methods including u.v.spectrophotometry and kinetic spectrophotometry have been reported [34-38]. Diode laser charge coupled device spectrometer for near infra red Raman spectroscopy has also been reported [39].
of Fujiwara reaction [40] reported for polyhalogenated hydrocarbons has been reported for the determination of pyridine. Other methods like Kjeldahl's methods have also been reported for pyridine determination [41, 42].

The most common spectrophotometric methods are based on Konig reaction which involves the reaction of pyridine with cyanogen bromide to form glutaronic aldehyde which is finally coupled with an aromatic amine to give a polymethine dye [43]. The various compounds reported as coupling reagents are aniline [44], benzidine [1], p-phenylene diamine [3], 2-naphthyl amine [45], p- methyl amino phenol [46], p-amino acetophenone [47], barbituric acid [48] anthranilic acid [49], 4-4 diaminostilbene, 2-2' disulphonate borate [50].

The sensitivity of the method using aniline is low and benzidine, 2-naphthyl amine and p-phenylene diamine are carcinogenic [51] and hence their use is not desirable. The methods using anthranilic acid is less sensitive.

In the present investigation a method has been developed for the determination of pyridine using 4-amino salicylic acid which is non-toxic and sensitive too. In this method pyridine reacts with cyanogen bromide to form glutaric aldehyde which is then condensed with 4-amino salicylic acid forming a yellow polymethine dye, measured at 400nm. The optimum reaction conditions and other important analytical parameters have been studied and also applied for the determination of pyridine in polluted water, aromatic hydrocarbons, laboratory and distillery waste.

**EXPERIMENTAL**

**Apparatus:**

A Carl Zeiss spectrophotometer with two matched silica cells of 1 cm path length were used for all spectral measurements. Calibrated glasswares were used for volumetric work.

For air sampling, midget impingers of 35ml capacity were used. Flow rate adjustable calibrated PIMCO make rotameter was used for measuring the flow rate.

**Reagents:**

**Standard pyridine solution:** A 1mg/ml solution of distilled pyridine was prepared in 1% glacial acetic acid in amber colour flask. Appropriate dilution of the above
solution gave a working standard solution of 10μg/ml. Working standard was prepared fresh daily.

**Cyanide solution:**
A 500 μg/ml aqueous solution of cyanide was prepared in distilled water.

**Bromine solution:**
A saturated aqueous solution of bromine was prepared and stored in an amber colour bottle.

**4-Amino salicylic acid:**
A 0.1% (w/v) solution in 20% aqueous ethanol was prepared.

**Sodium arsenite solution:**
A 1.5% (w/v) solution of sodium arsenite was prepared in distilled water.

All chemicals used were of analytical reagent grade or the best quality available and all solutions were prepared in double distilled water.

**Procedure:**

An aliquot of solution containing 2.5 to 20μg of pyridine was taken in a 10 ml calibrated flask and 1 ml solution of cyanide followed by 0.5 ml of saturated bromine water was added to it. The excess of bromine was decolourised by the dropwise addition of sodium arsenite solution and 2ml of 4-amino salicylic acid was added. The mixture was allowed to stand for 10 minutes for full colour development. The volume was made up to the mark and the yellow coloured polymethine dye having an absorption maxima at 400 nm was measured against distilled water as reference.

**Solvent Extraction:**

Aliquots of 100 ml containing 0.025 to 0.2 ppm (2.5 to 20 μg) of pyridine were taken in a separating funnel. The yellow coloured dye was obtained by the above procedure. The yellow dye was then extracted in 10ml of iso-amyl alcohol by taking two 5ml portions, after making the solution acidic by adding 5ml of 6M hydrochloric acid. The extract was dried over anhydrous sodium sulphate and the absorbance was measured at 400nm against iso-amyl alcohol as reference.
RESULTS AND DISCUSSIONS:

Spectral characteristics:

The absorption spectra of the yellow polymethine dye was scanned and is shown in Fig 3.1. It exhibits maximum absorbance at 400nm in aqueous as well as iso-amyl alcohol extract. The reagent blank shows negligible absorbance in this range.

The colour reaction:

The colour reaction is based on the König reaction [43], Pyridine reacts with cyanogen bromide [I] with heterocyclic cleavage of the ring and formation of glutaconic aldehyde (III) [52].

The glutaconic aldehyde so formed is condensed with 4-amino salicylic acid forming a yellow polymethine Schiff's base (IV) which is then measured colorimetrically for the determination of pyridine (Scheme-1).

Effect Of Variables:

\(p^H\):

The effect of \(p^H\) on the colour reaction was studied by taking known amounts of pyridine and developing the colour by varying the \(p^H\) of the solution (Table-1). It was found that the maximum absorbance and stability of the polymethine dye formed was at \(p^H\) 7-8. At lower or higher \(p^H\) the absorbance values decreased.

Time and Temperature:

The effect of time on the colour reaction was studied. It was found that the colour of the dye appeared after 1 minute but reached the maximum after 10 minutes. The dye was found to be stable for \(-15\) minutes (Fig.3.2).

The optimum temperature range for the complete colour reaction was found to be between 15-40°C. At higher or lower temperature absorbance values as well as stability of the dye decreased (Fig.3.3).

Reagent concentration:

Effect of various, reagents on the colour reaction were studied. It was found
SCHEME NO 1.
COLOUR REACTION

\[
\text{KCN} + \text{Br}_2 \rightarrow \text{KBr} + \text{CNBr}^+ \quad (\text{I})
\]

\[
\text{Pyridine} + \text{CNBr} \rightarrow \text{CNBr}^- \quad (\text{II})
\]

\[
\text{Pyridine} + 2\text{H}_2\text{O} \rightarrow \text{NHC} = \text{CHCH(OH)}\text{CHOH} + \text{NH}_2\text{CN} + \text{HBr} \quad (\text{III})
\]

Glutaconic aldehyde

\[
\text{OHC CHOH} + \text{H}_2\text{N} - \text{COOH} \rightarrow \text{HOOC-CH=CH-NH} \quad (\text{IV})
\]

Schiffs Base

Glutaconic aldehyde

4-Amino Salicylic acid
that 1-2 ml of 50(0) µg/ml of cyanide was sufficient to produce appropriate quantity of cyanogen bromide with 0.5 ml of bromine water (Fig. 3.4).

At least 0.5 ml of bromine water was essential to form appropriate quantity of cyanogen bromide. An excess of bromine caused no change in absorbance values, as the excess of bromine was removed by sodium arsenite solution.

The effect of sodium arsenite on the colour reaction was studied by varying amount of sodium arsenite and it was found that a minimum of 0.2 ml was necessary for the removal of excess of bromine and upto 1 ml of sodium arsenite caused no change in the absorbance value. The absorbance values decreased if more than 1 ml of sodium arsenite was used (Fig 3.5).

The study of the effect of 4-amino salicylic acid reveals that 2 to 4 ml of 4-amino salicylic acid caused no change in the absorbance value (Fig. 3.6).

**BEER'S LAW, SANDELL'S SENSITIVITY, MOLAR ABSORPTIVITY:**

The colour system was found to obey Beer's law in the range of 0.25 to 2 ppm (Fig 3.7) in the aqueous system and 0.025 to 0.2 ppm in the extractive system at 400 nm.

Molar absorptivity and Sandell’s sensitivity were calculated and were found to be 3.4x10³ litre mol⁻¹ cm⁻¹ (± 100) and 0.0023 µg/cm² respectively.

**REPRODUCIBILITY:**

The reproducibility of the colour reaction was checked by replicate analysis over a period of seven days. The standard deviation and relative standard deviation were found to be ± 0.008 and 1.92% respectively for 10 µg of pyridine in 10 ml of final volume (Table-2).

**EFFECT OF FOREIGN SPECIES:**

The effect of various foreign species likely to co-exist with pyridine in industrial effluents were studied by adding known amounts of different organic pollutants and inorganic ions to the test solution containing a total amount of 10 µg of pyridine per 10 ml of final volume (Table-3) prior to analysis. No interference was found for
phenol, p-cresol, benzene, formaldehyde, aniline, acetophenone etc.

APPLICATION OF THE METHOD:

The method has been successfully applied for the determination of pyridine in commercial benzene, alcohol, waste water, biological samples and air.

1. IN BENZENE:

Pyridine is found to be present in the crude coal tar distillate along with benzene. Since pyridine forms salts with acid it is easily extracted from the aromatic solvents with dilute hydrochloric acid. Complete extraction of traces of pyridine from other coal tar components like benzene, toluene and xylene was possible using hydrochloric acid.

So for determining the amounts of pyridine present in benzene, aliquots of C.P. benzene sample were extracted with 10ml of 1 M dilute hydrochloric acid in two portions of 5ml each. The extract was neutralized with dilute sodium hydroxide solution (1M) and then analysed by the above method as well as by the earlier reported method (49). The results are tabulated in Table-4.

2. IN ALCOHOL:

To check the recovery of pyridine from alcohol samples, known amount of pyridine was added to pyridine free alcohol samples. The mixture was then analysed by the recommended procedure. The recovery was found to be ~ 94%.

3. IN WASTE WATER:

The present method has been applied for the analysis of pyridine present in waste water collected from river Kharoon which receives effluents from Bhilai Steel Plant. The samples were analysed by spiking with known amount of pyridine. Aliquots of waste water sample were analysed by the recommended procedure and earlier reported method (49) and were found to contain 2 to 3.5 μg of pyridine and were in agreement. The results are tabulated in Table-4.

4. IN BIOLOGICAL SAMPLES (urine).

Samples of urine containing pyridine were not available, so known amount of
Pyridine was added to urine sample which was deproteinated by Trichloroacetic acid (53). This was then filtered and the filtrate was analysed by the recommended procedure and the earlier reported method (49). The recovery of pyridine was found to be ~ 95%.

5. IN AIR:

A modification of Wilson's procedure was used for determining amount of pyridine in air (54). Since standard air samples of pyridine were not available so known concentration of pyridine vapours were obtained by dropping a dilute solution of pyridine from a micro burette in a pre-heated closed chamber connected to an air sampling train.

Air containing pyridine vapours were drawn at the rate of 0.250 litres/minutes for 15 minutes through two midget impingers connected in series, each containing 10ml of 4.4% glacial acetic acid solution forming a part of the sampling train. Glacial acetic acid (4.4% in distilled water) solution was used as absorbing solution for pyridine (55). After sampling aliquots of this solution were taken and 1ml of cyanide solution followed by 0.5 ml of saturated bromine water was added to it. The excess of bromine was decolourised by dropwise addition of sodium arsenite solution and 2ml of 4-amino salicylic acid was added. The mixture was allowed to stand for 10 minutes for full colour development. The volume was made upto the mark and the absorbance was measured at 400nm. A reagent blank was prepared similarly. It was found that 100% absorption of pyridine takes place in the first impinger itself. The results are tabulated in Table 5 and 6.

CONCLUSION:

The method is fast, selective and sensitive. Carcinogenic compounds are not used in the present method. The method has been compared with other spectro photometric method (Table-7) and found to be sensitive and faster than most of the reported methods. The reagent used is stable and easily available.

A Kinetic method (50) using 4-4'diamino stilbene 2-2' disulphonate borate, though more sensitive suffers from the disadvantage that, it uses a reagent which is unstable and not easily available.

The present method can be used for determination of pyridine in alcohol, benzene, air and biological samples (urine).
FIG 3: ABSORPTION SPECTRA OF THE DYE AND REAGENT BLANK

A. CONCENTRATION OF PYRIDINE = 10 µg/10 ml
B. CONCENTRATION OF PYRIDINE = 5 µg/10 ml.
C. REAGENT BLANK.
**FIG 3.2: EFFECT OF TIME ON THE COLOUR DEVELOPMENT.**
CONCENTRATION OF PYRIDINE = 10µg / 10ml.

**FIG 3.3: EFFECT OF TEMPERATURE ON THE COLOUR DEVELOPMENT**
CONCENTRATION OF PYRIDINE = 10µg / 10ml.
FIG. 3.4 EFFECT OF CYANIDE ON COLOUR REACTION.
CONCENTRATION OF PYRIDINE = 10μg/10ml

FIG. 3.5 EFFECT OF SODIUM ARSENITE ON COLOUR REACTION.
CONCENTRATION OF PYRIDINE = 10μg/10ml
FIG. 3: EFFECT OF 4-AMINO SALICYLIC ACID ON COLOUR REACTION.
CONCENTRATION OF PYRIDINE = 10 µg/10 mL.

FIG. 4: CALIBRATION CURVE FOR THE DETERMINATION OF PYRIDINE IN AQUEOUS SYSTEM.
FIG. 3: CALIBRATION CURVE FOR THE DETERMINATION OF PYRIDINE IN EXTRACTIVE SYSTEM.
Table - 1.

Effect of $p^N$:
Concentration of pyridine = 10 $\mu g$ / 10ml (1ppm).

<table>
<thead>
<tr>
<th>$p^N$</th>
<th>Absorbance at 400nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.0</td>
<td>0.215</td>
</tr>
<tr>
<td>5.5</td>
<td>0.300</td>
</tr>
<tr>
<td>6.0</td>
<td>0.355</td>
</tr>
<tr>
<td>6.5</td>
<td>0.400</td>
</tr>
<tr>
<td>7.0</td>
<td>0.435</td>
</tr>
<tr>
<td>7.5</td>
<td>0.435</td>
</tr>
<tr>
<td>8.0</td>
<td>0.435</td>
</tr>
<tr>
<td>8.5</td>
<td>0.405</td>
</tr>
<tr>
<td>9.0</td>
<td>0.295</td>
</tr>
<tr>
<td>9.5</td>
<td>0.280</td>
</tr>
<tr>
<td>10.0</td>
<td>0.250</td>
</tr>
</tbody>
</table>

* Mean of three repetitive analyses.

Table - 2

Reproducibility of the Method
Concentration of Pyridine = 10 $\mu g$ / 10ml (1ppm).

<table>
<thead>
<tr>
<th>No. of days</th>
<th>Absorbance at 400nm*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.41</td>
</tr>
<tr>
<td>2</td>
<td>0.43</td>
</tr>
<tr>
<td>3</td>
<td>0.42</td>
</tr>
<tr>
<td>4</td>
<td>0.415</td>
</tr>
<tr>
<td>5</td>
<td>0.41</td>
</tr>
<tr>
<td>6</td>
<td>0.42</td>
</tr>
<tr>
<td>7</td>
<td>0.405</td>
</tr>
</tbody>
</table>

Mean = 0.415
Standard deviation = ($\pm$) 0.008
Relative standard deviation = 1.92%

* Mean of three repetitive analyses.
Table - 3.

Effect of Foreign species on colour reaction:
Concentration of pyridine = 2 ppm.

<table>
<thead>
<tr>
<th>Foreign species</th>
<th>Tolerance limit * (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetone</td>
<td>11000</td>
</tr>
<tr>
<td>Acetophenone</td>
<td>10000</td>
</tr>
<tr>
<td>Formaldehyde</td>
<td>9000</td>
</tr>
<tr>
<td>Benzene, Thiophene</td>
<td>2000</td>
</tr>
<tr>
<td>Ethanol, Methanol</td>
<td>1200</td>
</tr>
<tr>
<td>Phenol</td>
<td>1000</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>900</td>
</tr>
<tr>
<td>Aniline, p-cresol</td>
<td>800</td>
</tr>
<tr>
<td>SO$_4^{2-}$, CH$_3$COO$^-$,Cl$^-$</td>
<td>500</td>
</tr>
<tr>
<td>Na$^+$, K$^+$</td>
<td>500</td>
</tr>
<tr>
<td>Cu$^{2+}$</td>
<td>350</td>
</tr>
<tr>
<td>Fe$^{2+}$, Fe$^{3+}$</td>
<td>275</td>
</tr>
<tr>
<td>Ni$^{2+}$</td>
<td>250</td>
</tr>
</tbody>
</table>

* Amount of foreign species that causes a (±) 2% change in absorbance.
Table - 4.
Determination of pyridine in various samples of Benzene and waste water:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Pyridine Originally* Found (μg)</th>
<th>Pyridine* Added (μg)</th>
<th>Total Pyridine found* (μg)</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(A)</td>
<td>(B)</td>
<td>(A)</td>
<td>(B)</td>
</tr>
<tr>
<td>S₁</td>
<td>4.35</td>
<td>4.25</td>
<td>5</td>
<td>9.42</td>
</tr>
<tr>
<td></td>
<td>4.5</td>
<td>4.45</td>
<td>10</td>
<td>14.55</td>
</tr>
<tr>
<td></td>
<td>4.30</td>
<td>4.28</td>
<td>20</td>
<td>24.45</td>
</tr>
<tr>
<td>S₂</td>
<td>3.0</td>
<td>2.90</td>
<td>5</td>
<td>8.15</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>2.42</td>
<td>10</td>
<td>12.67</td>
</tr>
<tr>
<td></td>
<td>3.05</td>
<td>3.00</td>
<td>20</td>
<td>23.10</td>
</tr>
<tr>
<td>S₃</td>
<td>3.20</td>
<td>3.00</td>
<td>5</td>
<td>8.32</td>
</tr>
<tr>
<td></td>
<td>3.40</td>
<td>3.20</td>
<td>10</td>
<td>13.44</td>
</tr>
<tr>
<td></td>
<td>2.90</td>
<td>2.85</td>
<td>20</td>
<td>22.95</td>
</tr>
</tbody>
</table>

S₁, S₂ - samples of Benzene, S₃ - sample of waste water.
* Mean of three repetitive analyses; Volume of sample taken = 2ml
(A) proposed method; (B) = Reference method [49]
## ANALYSIS OF PYRIDINE IN LABORATORY AIR

### Table - 5

Effect of flow rate on absorption efficiency:
Concentration of acetic acid = 4.4%.
Sampling time = 15 minutes.
Flow rate = litre / min.

<table>
<thead>
<tr>
<th>Amount of Pyridine (μg)</th>
<th>Flow rate (litre / min)</th>
<th>0.100</th>
<th>0.250</th>
<th>0.750</th>
<th>1.00</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(a)</td>
<td>(a)</td>
<td>(a)</td>
<td>(a)</td>
</tr>
<tr>
<td>5</td>
<td>4.5</td>
<td>90.0</td>
<td>4.95</td>
<td>99.0</td>
<td>4.85</td>
</tr>
<tr>
<td>10</td>
<td>9.2</td>
<td>92.0</td>
<td>9.91</td>
<td>99.1</td>
<td>9.82</td>
</tr>
<tr>
<td>20</td>
<td>18.5</td>
<td>92.5</td>
<td>19.80</td>
<td>99.0</td>
<td>19.05</td>
</tr>
</tbody>
</table>

a = amount of pyridine found (μg); b = % absorbed.

### Table - 6.

Effect of time on absorption efficiency.
Flow rate = 0.250 litre / min.
Concentration of acetic acid = 4.4%.

<table>
<thead>
<tr>
<th>Amount of Pyridine (μg)</th>
<th>Sampling Time (in minute)</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>35</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>a</td>
<td>b</td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>5</td>
<td>3.50</td>
<td>70.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>6.68</td>
<td>66.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>14.98</td>
<td>74.9</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a = amount of pyridine found (μg); b = % absorbed.
Table - 7

Comparison of some spectrophotometric method for determination of Pyridine:

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Reagents (Ref)</th>
<th>( \lambda_{max} ) (nm)</th>
<th>Colour</th>
<th>( pH )</th>
<th>Time for full colour development</th>
<th>Lowest determination limit (ppm)</th>
<th>Remarks.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Benzidine[1]</td>
<td>520</td>
<td>Red</td>
<td>6.8-8.</td>
<td>210 minutes</td>
<td>0.005</td>
<td>Carcinogenic</td>
</tr>
<tr>
<td>2</td>
<td>P-Phenylenediamine[3]</td>
<td>480</td>
<td>Intense Orange</td>
<td>5</td>
<td>30 minutes</td>
<td>10</td>
<td>Carcinogenic</td>
</tr>
<tr>
<td>3</td>
<td>Barbituric acid</td>
<td>578</td>
<td>Violet</td>
<td>5</td>
<td>50 minutes</td>
<td>2</td>
<td>Requirs longer time for colour development</td>
</tr>
<tr>
<td>4</td>
<td>Sulphanilic acid[34]</td>
<td>465</td>
<td>Yellow</td>
<td>7.5</td>
<td>50-90 minutes</td>
<td>0.4</td>
<td>Longer time for colour development</td>
</tr>
<tr>
<td>5</td>
<td>Anthranilic acid[49]</td>
<td>470</td>
<td>Yellow</td>
<td>7.5</td>
<td>10 minutes</td>
<td>0.4</td>
<td>Less sensitive</td>
</tr>
<tr>
<td>6</td>
<td>4,4'-diaminstibene 2,2'-disulphonate borate [50]</td>
<td>490</td>
<td>Orange</td>
<td>8.5</td>
<td>30 second</td>
<td>0.01</td>
<td>Kinetic method though more sensitive, reagent unstable and not easily available.</td>
</tr>
<tr>
<td>7</td>
<td>4 Aminosalicylic acid (Proposed Method)</td>
<td>400</td>
<td>Yellow</td>
<td>7-8</td>
<td>10 minutes</td>
<td>0.025</td>
<td>Sensitive, fast and reagent easily available</td>
</tr>
</tbody>
</table>
REFERENCE

43. W. N. Aldridge, Analyst, 70, (1945), 474.