CHAPTER IV

SPECTROPHOTOMETRIC DETERMINATION OF
AMINO GROUP CONTAINING DRUGS
4.1 INTRODUCTION :

1. 5-Aminosalicylic acid (Mesalamine)

Sulfasalazine is effective in treatment for maintenance of clinical remission in active ulcerative colitis. It is composed of sulfapyridine in diazo linkage with 5-aminosalicylic acid. In colon, sulfasalazine is almost completely split into 5-aminosalicylic acid and sulfapyridine by bacterial azoreductase. Mesalamine is reported to be an active metabolite of sulfasalazine. Therefore, it is preferred to sulfasalazine for ulcerative colitis.

It is estimated by titrimetric, photometric, spectrofluorometric and HPLC techniques.

2. p-Amino benzoic acid

4-Amino benzoic acid (PABA) is widely used as sun screen agent and as a B-complex factor. Various reported procedures for the estimation include titrimetric, UV spectrophotometric, colorimetric, refractometric, fluorimetric, phosphorimetric, gravimetric, microbiological and chromatographic techniques.

3. p-Amino Salicylic acid

The development of effective chemotherapeutic agents for the treatment of tuberculosis began in 1938, when it was observed that sulphanilamide had a slight inhibitory effect in the course
of experimental tuberculosis in guinea pigs. Dapsone (DDS) was investigated clinically, but was considered to be toxic. Major early advances in the chemotherapy of tuberculosis were the discovery of streptomycin, p-aminosalicylic acid and isoniazid. Later, the antitubercular properties of ethambutol and semisynthetic antibiotic rifampicin were discovered.

Until recently, p-aminosalicylic acid was considered a first line drug for the chemotherapy of tuberculosis and included in combination regimens with isoniazid and streptomycin. Most commonly used triple drug combination, in the past for initial treatment contained streptomycin (1g/day i.m.), isoniazid (300-800 mg p.o.) and p-aminosalicylic acid (12-15 mg p.o. or by i.v. infusion) for 30-90 days.

p-Aminosalicylic acid is taken orally, usually in tablet form. It is bacteriostatic. The antimicrobial activity of p-aminosalicylic acid is highly specific for M. tuberculosis.

Various titrimetric, spectrophotometric, radiometric, refractometric and fluorimetric techniques have been suggested for the determination of p-aminosalicylic acid. The pharmacopoeial method, for the estimation of this
drug and its dosage forms include titrimetric and UV spectrophotometric procedures. The former is tedious and time consuming and the later is non specific.

4. Procaine Hydrochloride

Local anaesthetics are drugs which block reversibly the generation and the conduction of impulses along a nerve fibre. They are used to abolish the pain sensation in restricted areas of the body. The first well documented formulation that allowed us to identify a chemical entity was given by Dioscorides, who recommended a preparation from roses for topical application. In this formulation, 2-phenylethanol has been found to have local anaesthetic properties. Its action results from their ability to depress impulses from afferent nerves of the skin, surface mucosa and muscle. These agents are widely used especially in surgery, dentistry and ophthalmology.

Procaine, benzocaine, lignocaine, amethocaine and their salts are widely used as local anaesthetics. Among them, procaine hydrochloride, 2-diethylaminoethyl p-aminobenzoate monohydrochloride was the first synthetic local anaesthetic agent to be introduced in therapy. It does not have the severe local and systemic toxicity of cocaine. Procaine hydrochloride is not effective on intact skin or mucous membranes. The action may be prolonged by the concurrent administration of epinephrine or other vasoconstrictors.
Various titrimetric, UV spectrophotometric, visible spectrometric fluorimetric, phosphorimetric, polarographic, paper chromatographic, thin layer chromatographic, gas liquid chromatographic and high pressure liquid chromatography techniques have been suggested for the determination of local anaesthetics. The pharmacopoeial method for the estimation of the drug and its dosage forms include titrimetric and UV spectrophotometric procedures.

5. Aminophylline

Purines are widely scattered in animal and plant kingdom. Some of them have diuretic and antiasthmatic properties.

Aminophylline is a salt of theophylline and ethylenediamine. It plays an important role in the management of the asthmatic conditions. It is a smooth muscle relaxant often incorporated in medication for acute bronchospasm attacks. It is also effective as diuretic when given by the intravenous route.

Various titrimetric, spectrophotometric, radiometric techniques are suggested for its determination.
In present work, a spectrophotometric method based on the reaction of primary amino group with acetylacetone-formaldehyde reagent in aqueous medium is described. The effects of various reaction conditions such as concentration of reagent and drug, time and temperature of reaction, pH, etc. on color development are investigated.

Pure samples of mesalamine, p-amino benzoic acid, p-aminosalicylic acid, procaine hydrochloride and amino-phylline are analysed by the proposed method. The results compare favorably with those obtained by pharmacopoeial method.127,150

The procedure is applied successfully to the analysis of these drugs in combination with other drugs in their dosage forms. The method is simple, rapid, precise and accurate.
4.2 EXPERIMENTAL

4.2.1 Apparatus

As described under 1.2.1.

4.2.2 Reagents and Materials

5-Aminosalicylic acid (Sun Pharma.), p-aminobenzoic acid (IP), p-aminosalicylic acid (IP), procaine HCl (BP), aminophylline (IP), Formaldehyde (SD's), Acetylacetone (SD's), Sodium acetate (anhydrous) (BDH), Acetic acid (SD's) and double distilled water were used in the study.

The dosage forms of drugs were procured from local market.

4.2.2.1 Preparation of Buffer solution

As described under 1.2.2.2.

4.2.2.2 Preparation of reagent solution

As described under 1.2.2.3.

4.2.2.3 Preparation of Standard 5-Aminosalicylic acid solution

5-Aminosalicylic acid (20mg) was weighed accurately and dissolved in 0.1ml concentrated HCl and diluted to 100ml with water.
4.2.2.4 Preparation of Standard p-Aminobenzoic acid solution
p-Aminobenzoic acid (30mg) was weighed accurately and dissolved in 5ml ethanol and diluted to 100ml with water.

4.2.2.5 Preparation of Standard Sodium p-Aminosalicylate solution
Sodium p-aminosalicylate (30mg) was weighed accurately and dissolved in and diluted to 100ml with water.

4.2.2.6 Preparation of Standard Procaine hydrochloride solution
Procaine hydrochloride (150mg) was weighed accurately and dissolved in and diluted to 100ml with water.

4.2.2.7 Preparation of Standard Aminophylline solution
Aminophylline (114mg) was weighed accurately and dissolved in and diluted to 100ml with water.

Freshly prepared solution was used in the study.

4.2.3 Procedure
5-Aminosalicylic acid
4.2.3.1 Determination of wavelength of maximum absorbance
Standard 5-aminosalicylic acid solution (2.0ml) was pipetted into a 100ml conical flask. The reagent solution
(5.0ml) was added to it and the reaction mixture was immersed in a boiling water bath for 20 min. It was cooled to room temperature. The contents of the flask were transferred quantitatively to 25ml volumetric flask with the help of water. The volume was adjusted to the mark with water. The absorbance of the colored solution was scanned in the range of 350 to 550 nm against blank.

The blank was prepared similarly in which volume of standard 5-aminosalicylic acid solution was replaced by an equal volume of water.

Maximum absorbance was obtained at 415 nm (Fig.7).

p-Aminobenzoic acid, p-aminosalicylic acid, procaine hydrochloride and aminophyllin were treated similarly. The maximum absorbance was obtained at 415 nm with all the drugs.

4.2.3.2 Lambert-Beer's curve for 5-aminosalicylic acid

Standard 5-aminosalicylic acid solution (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0ml) was pipetted into a series of 100ml conical flask and treated as described under 4.2.3.1.

The absorbance of the reaction mixture was measured at 415 nm against the reagent blank (Fig.8).
The Lambert-Beer's concentration range for 5-aminosalicylic acid, p-aminobenzoic acid, sodium p-aminosalicylate, procaine hydrochloride and aminophylline are given in Table VIII.

4.2.4 Factors affecting the development of color

4.2.4.1 Effect of concentration of reagent

Standard 5-aminosalicylic acid solution (2.0ml) was treated with different volumes of the reagent. The reaction mixture was immersed in a boiling water bath for 20 min. and treated as described under 4.2.3.1. The absorbance of the colored solution was measured at 415 nm against the reagent blank.

Maximum color intensity was obtained in the presence of 3.0ml of reagent solution which remains constant on further increasing the reagent concentration (Fig.9). In the present study, 5.0ml of reagent is used.

4.2.4.2 Effect of temperature on reaction

The reaction of standard 5-aminosalicylic acid (2.0ml) with reagent solution (5.0ml) was carried out at 25°, 37°, 60° and 100°C (boiling water bath) for 20 min. as described under 4.2.3.1. The absorbance of the reaction mixture was measured at 415 nm against the reagent blank (Fig.10).
Maximum color intensity was obtained when the reaction is carried out at 100° (boiling water bath).

4.2.4.3 Time of reaction

The standard 5-aminosalicylic acid solution (2.0ml) was mixed with the reagent solution (5.0ml). The reaction mixture was immersed in boiling water both for different time intervals and treated as described under 4.2.3.1. The absorbance of colored solution was measured at 415 nm against the reagent blank.

Maximum color intensity was obtained after 20 min. heating which remained constant on further heating. (Fig.11).

4.2.4.4 Stability of colored product

5-Aminosalicylic acid solution (2.0ml) was reacted with the reagent solution (5.0ml) as described under 4.2.3.1. The color intensity was measured at 415 nm after storage for different time intervals (Fig.12). The color remains stable for at least 5 hrs.

Factors 4.2.4.1 to 4.2.4.4 were also studied for remaining drugs.
4.2.5 Analysis of the drugs

4.2.5.1 Analysis of 5-Aminosalicylic acid

5-Aminosalicylic acid (20.0mg) was weighed accurately and dissolved in 0.1ml concentrated hydrochloric acid and diluted to 100ml with water. The solution was analysed as described under 4.2.3.1.

The amount of 5-aminosalicylic acid was determined by referring to the standard curve (Table IX).

4.2.5.2 Analysis of p-aminobenzoic acid

p-Aminobenzoic acid (30.0mg) was weighed accurately and dissolved in ethanol (5.0ml) and diluted to 100ml with water. The solution was analysed as described under 4.2.3.1.

The amount of p-aminobenzoic acid was determined by referring to the standard curve (Table IX).

4.2.5.3 Analysis of sodium p-aminosalicylate

Sodium p-aminosalicylate (30mg) weighed accurately and dissolved in and diluted to 100ml with water. The solution was analysed as described under 4.2.3.1.

The amount of sodium p-aminosalicylate was determined by referring to the standard curve (Table IX).
4.2.5.4 Analysis of procaine hydrochloride

Procaine hydrochloride (150.0mg) was weighed accurately and dissolved in and diluted to 100ml with water. The solution was analysed as described under 4.2.3.1.

The amount of procaine hydrochloride was determined by referring to the standard curve (Table IX).

4.2.5.5 Analysis of Aminophylline

Aminophylline (114.0mg) was weighed accurately and dissolved in and diluted to 100ml with water. The solution was analysed as described under 4.2.3.1.

The amount of aminophylline was determined by referring to the standard curve (Table IX).

4.2.5.6 Analysis of 5-aminosalicylic acid tablets

Twenty tablets were weighed and powdered. The powder equivalent to ca. 20mg 5-aminosalicylic acid was weighed accurately and mixed with concentrated HCl (0.1ml) and water (75ml). The mixture was shaken occasionally and filtered through Whatman No.41 filter paper. The residue was washed thoroughly with water. The filtrate and the washings were combined and diluted to 100ml with water. The solution (2.0ml) was analysed as described under 4.2.5.1.
4.2.5.7 Analysis of procaine hydrochloride injection

The solution of injection was diluted suitably with water to contain procaine hydrochloride equivalent to 1.5mg/ml and analysed as described under 4.2.5.4.

4.2.5.8 Analysis of aminophylline tablets

Twenty tablets were weighed and powdered. The powder equivalent to ca.114.0mg aminophylline was weighed accurately and mixed with water (75ml). The mixture was shaken occasionally and filtered through Whatman No.41 filter paper. The residue was washed thoroughly with water. The filtrate and the washings were combined and diluted to 100ml with water. The solution (2.0ml) was analysed as described under 4.2.5.5.

4.2.5.9 Analysis of aminophylline in combination with dried aluminium hydroxide gel and phenobarbitone tablet

Twenty tablets were weighed and powdered. The powder equivalent to aminophylline (ca.114.0mg) was weighed accurately and mixed with water (75ml). The mixture was shaken occasionally as filtered through Whatman No.41 filter paper. The residue was washed thoroughly with water. The filtrate and the washings were combined and diluted to 100ml with water. The solution (2.0ml) was analysed as described under 4.2.5.5.
4.2.5.10 Analysis of aminophylline injection

The solution for injection was diluted suitably with water to contain aminophylline equivalent to 1.14mg/ml. The solution (2.0ml) was analysed as described under 4.2.5.5.
4.3 RESULTS AND DISCUSSION

Various colorimetric procedures are reported for the estimation of primary amino group containing drugs. Most of them involve diazotization of primary aromatic amino group by reaction with nitrous acid, followed by coupling reaction. This reaction is not suitable for the aliphatic primary amines.

The proposed method is based on the reaction between primary amino group and acetylacetone-formaldehyde reagent to give yellow colored products. Various parameters of reaction are standardized to obtain maximum color intensity (Fig.9-12). When solution of drug was reacted with buffered acetylacetone-formaldehyde reagent for 20 min in boiling water bath, the yellow colored product formed has wavelength of maximum absorbance at 415 nm. The color is stable for more than 5 hours.

The Lambert-Beer's concentration range, molar absorptivity, Sandell's sensitivity and standard deviation (n=9) for each drug are evaluated (Table VIII and IX). The slopes, intercepts and correlation co-efficients obtained by linear regression analysis of data are recorded in Table VIII. The results show that the method is precise, accurate and rapid.
Pure samples of p-aminobenzoic acid, Sodium p-amino-
salicylate, procaine hydrochloride and aminophylline and their
pharmaceutical dosage forms are analysed by proposed procedure.
The results are in good agreement with those obtained by
pharmacopoeial method (Table X).

The proposed procedure was applied for the assay of
aminophylline in combination with phenobarbitone and dried
aluminium hydroxide gel in tablets. The presence of pheno-
barbitone and dried aluminium hydroxide gel as well as usual
diluents and lubricants employed in the formulation of dosage
forms do not interfere in the proposed method.
Figure 7: Visible spectrum of the colored products obtained on reacting 5-Aminosalicylic acid with Acetylacetone-Formaldehyde reagent.
Figure 8: Lambert-Beer's curve for 5-Aminosalicylic acid.
Figure 9: Effect of concentration of reagent solution.
Figure 10: Effect of temperature on color intensity.
Figure 11: Effect of time of heating on color intensity.
Figure 12: Effect of time on stability of colored product.
<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Name of Drug</th>
<th>Beer's law limit mcg/ml</th>
<th>Molar absorptivity 1mol⁻¹ cm⁻¹ x 10³</th>
<th>Sandell's sensitivity mcg cm⁻²/0.001A</th>
<th>Slope x 10⁻¹</th>
<th>Intercept</th>
<th>Corr. Coeff</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>5-Aminosalicylic acid</td>
<td>4-32</td>
<td>4.354</td>
<td>0.0351</td>
<td>0.0280</td>
<td>0.0029</td>
<td>0.9999</td>
</tr>
<tr>
<td>2.</td>
<td>p-Aminobenzoic acid</td>
<td>6-48</td>
<td>2.500</td>
<td>0.0500</td>
<td>0.0196</td>
<td>0.0070</td>
<td>0.9999</td>
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<td>3.</td>
<td>Sodium p-aminosalicylate</td>
<td>6-48</td>
<td>3.270</td>
<td>0.0468</td>
<td>0.0213</td>
<td>0.0011</td>
<td>0.9999</td>
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<td>4.</td>
<td>Procaine HCl</td>
<td>30-270</td>
<td>0.660</td>
<td>0.4128</td>
<td>0.0024</td>
<td>0.0102</td>
<td>0.9997</td>
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<td>5.</td>
<td>Aminophylline</td>
<td>45-182</td>
<td>1.533</td>
<td>0.2976</td>
<td>0.0033</td>
<td>0.0022</td>
<td>0.9997</td>
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<tr>
<td>Sr.No.</td>
<td>Name of drug</td>
<td>% Recovery ± SD&lt;sup&gt;a&lt;/sup&gt; by Proposed Method</td>
<td>Pharmacopoeial Method&lt;sup&gt;127,150&lt;/sup&gt;</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>1</td>
<td>5-Aminosalicylic acid</td>
<td>99.65 ± 0.75</td>
<td>-</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>2</td>
<td>p-Aminobenzoic acid</td>
<td>100.12 ± 0.88</td>
<td>99.85 ± 0.80</td>
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<tr>
<td>3</td>
<td>Sodium p-aminosalicylate</td>
<td>99.87 ± 0.69</td>
<td>99.50 ± 0.75</td>
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<tr>
<td>4</td>
<td>Procaine HCl</td>
<td>98.55 ± 0.85</td>
<td>99.05 ± 0.90</td>
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<tr>
<td>5</td>
<td>Aminophylline</td>
<td>97.20 ± 0.75</td>
<td>98.10 ± 0.98</td>
<td></td>
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<sup>a</sup> Standard deviation was calculated from the results of nine determinations.
TABLE X
ANALYSIS OF DRUGS AND THEIR DOSAGE FORMS

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Formulation</th>
<th>Labelled amount</th>
<th>% Recovery by Proposed Method</th>
<th>% Recovery by Pharmacopoeial Method</th>
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<tr>
<td>1</td>
<td>POWDER</td>
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<td></td>
<td></td>
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<tr>
<td>(i)</td>
<td>5-Aminosalicylic acid</td>
<td></td>
<td>98.54</td>
<td></td>
</tr>
<tr>
<td>(ii)</td>
<td>p-Aminobenzoic acid</td>
<td></td>
<td>98.75</td>
<td>98.80</td>
</tr>
<tr>
<td>(iii)</td>
<td>Sodium-p-aminosalicylate</td>
<td></td>
<td>98.48</td>
<td>97.89</td>
</tr>
<tr>
<td>(iv)</td>
<td>Procaine HCl</td>
<td></td>
<td>98.93</td>
<td>99.12</td>
</tr>
<tr>
<td>(v)</td>
<td>Aminophylline</td>
<td></td>
<td>89.83</td>
<td>89.65</td>
</tr>
<tr>
<td>2</td>
<td>TABLETS</td>
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<td></td>
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</tr>
<tr>
<td>(i)</td>
<td>5-Aminosalicylic acid</td>
<td>400mg</td>
<td>99.85</td>
<td>98.89</td>
</tr>
<tr>
<td>(ii)</td>
<td>Aminophylline</td>
<td>100mg</td>
<td>89.38</td>
<td>90.44</td>
</tr>
<tr>
<td>(iii)</td>
<td>Aminophylline</td>
<td>200mg</td>
<td>91.55</td>
<td>90.87</td>
</tr>
<tr>
<td>(iv)</td>
<td>Dried Aluminium hydroxide gel</td>
<td>250mg</td>
<td></td>
<td></td>
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<td>(v)</td>
<td>Phenobarbitone</td>
<td>16mg</td>
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<td>3</td>
<td>INJECTIONS</td>
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<tr>
<td>(i)</td>
<td>Procaine HCl</td>
<td>2% w/v</td>
<td>103.90</td>
<td>102.10</td>
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<tr>
<td>(ii)</td>
<td>Aminophylline</td>
<td>2.5% w/v</td>
<td>92.14</td>
<td>93.25</td>
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\(a = \) Average value of five determination.