CHAPTER - II

SPECTROPHOTOMETRIC DETERMINATION OF SULPHONAMIDES
2.1 INTRODUCTION

The modern chemotherapy began with the introduction of sulphonamides in clinic. They are the first effective chemotherapeutic agents to be employed systematically.

Sulphanilamide represents the basic skeleton of sulphonamides. It was first synthesised in 1908. In 1937, it was proved to be a drug when it was established that it gets released in the body from prontosil by the metabolic cleavage of the azo linkage. This discovery led to the synthesis of number of congeneric substances known as sulphonamides. Only few of them have retained an established place in therapy. Due to introduction of penicillins in 1945, the interest has shifted gradually towards the antibiotics. Factors such as toxicity and development of resistance by microorganisms have reversed the interest of scientists from antibiotics to sulphonamides. It resulted in the synthesis of new sulphonamides with improved physiological properties and thus, started new phase for sulphonamides from 1957.

The term sulphonamides is usually employed as a generic name for the derivatives of p-amino-
benzene sulphonamides. The presence of free primary aromatic amino group is essential for antibacterial activity. Pharmacologically, all the sulphonamides are similar, however, they differ in their solubility and protein binding behaviour and hence they have different rate of absorption and excretion.

The therapeutic effect of sulphonamides is achieved by stagnating the growth and multiplication of the infectious organisms. This allows host to combat the infection by its cellular and humoral defence mechanism. In usual doses, sulphonamides exhibit bacteriostatic effect, however, in high doses bactericidal action is also observed. They possess broad spectrum activity against both gram-positive and gram-negative bacteria, such as Mycobacterium tuberculosis, Streptomyces pneumoniae, Haemophilus influenzae, Corynebacterium diphtheriae, Nocardia, Actinomyces, Escherichia coli, Meningococci, etc.

Sulphonamides exhibit variety of therapeutic applications. Their main uses include treatment of urinary tract infections (sulphafurazole), bacillary dysentery (sulphaguanidine), ulcerative colitis (sulphasalazine), meningococcal meningitis (sulphadiazine), nocardiosis (sulfisoxazole), con-
junctivities and other eye infections (sulphacetamide), chloroquine resistant malarial parasite (sulphadoxine), diabetes (tolbutamide), burn therapy (mafenide acetate), etc. It is also used in combination with other drugs for specific treatment e.g. for urinary tract and respiratory tract infections (sulphamethoxazole and trimethoprim), for prophylactic antimalarial treatment (sulphadoxine and pyrimethamine), etc.

De Reeder has published excellent reviews on methods for determination of sulphonamides. Various titrimetric, ultraviolet spectrophotometric, visible spectrophotometric, atomic absorption spectrophotometric, polarographic, differential scanning calorimetric and microbiological methods have been described.

Titration with sodium nitrite solution to determine the aromatic amino function in acidic medium is the most widely used assay procedure for sulphonamides and their dosage forms in the official compendia.

Among colorimetric procedures, the popular and widely used method is the Bratton-Marshall method in which diazotised sulphonamide is coupled with N-(1-naphthyl)ethylenediamine di-
hydrochloride to give intense pink color. Dux and Rosenblum have studied the method critically.

Chromatographic procedures are preferentially employed for the separation, identification and estimation of these drugs. The subject have been reviewed extensively. Paper chromatographic, thin layer chromatographic and gas-liquid chromatographic techniques are also described for the determination of sulphonamides. Gas chromatographic procedures required prior derivatisation of sulphonamides which is usually a time consuming step. GLC is preferred to TLC because of its greater precision and resolution power. HPLC is the most popular procedure for the sulphonamides separation and quantification.

Sawicki et al. reported spectrophotometric procedure for the estimation of aromatic amines. This is based on the oxidative coupling reaction of aromatic amines with 3-methyl-2-benzothiazolinone hydrazone (MBTH) to yield highly colored condensation products. It was, therefore, thought of interest to extend the applicability of MBTH reagent to the determination of sulphonamides in bulk drug and in their dosage forms.
In present work, conditions of the reaction are modified suitably to estimate sulphonamides. The effect of various parameters of the reaction, such as concentration of MBTH, concentration of ferric chloride, concentration of sulphonamides and time for the color development are optimised. The method is applied successfully to assay sulphamethoxypyridazine, sulphamerazine, sulphaguanidine, sulphasoxazole, sulphasphenazole and phthalylsulphathiazole in bulk powder as well as in their pharmaceutical dosage forms.
2.2 EXPERIMENTAL

2.2.1 Apparatus

1. Double beam Beckman Model 25 spectrophotometer having matched cells of 1 cm length path.

2. Corning volumetric flasks of 10 ml, 50 ml and 100 ml capacity.

2.2.2 Reagents and Materials

Sulphamethoxypyridazine B.P., Sulphaphenazole I.P., Sulphamerazine U.S.P., Sulphamethoxazole I.P., Sulphaguanidine I.P., Phthalylsulphathiazole I.P., 3-Methyl-2-Benzothiazolinone hydrazone hydrochloride (Sigma, U.S.A.), Ferric chloride (BDH), Hydrochloric acid B.P. and double distilled water were used in the study.

The dosage forms of the various drugs were procured from local market.

2.2.2.1 Preparation of solution of 3-Methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH)

The solution of MBTH was prepared by dissolving 3-methyl-2-benzothiazolinone hydrazone hydrochloride (500 mg) in and diluted to 100 ml with water.
2.2.2.2 Preparation of Ferric chloride solution

Ferric chloride (1 gm) was dissolved in hydrochloric acid solution (0.1 M) and diluted to 100 ml with the same solvent.

2.2.2.3 Preparation of standard Sulphamethoxypyridazine, Sulphaphenazole, Sulphamerazine or Sulphaguanidine solution

Sulphamethoxypyridazine, Sulphaphenazole, Sulphamerazine or Sulphaguanidine (40 mg) was weighed accurately and dissolved in and diluted to 100 ml with 0.1 M hydrochloric acid. An aliquot (10 ml) was diluted further to 50 ml with the same solvent. Final solution contained 80 mcg of the drug per ml of the solution.

2.2.2.4 Preparation of standard Sulphamethoxazole solution

Sulphamethoxazole (20 mg) was weighed accurately and dissolved in 0.1 M hydrochloric acid. The final volume was adjusted to 100 ml with the same solvent.

2.2.2.5 Preparation of standard Phthalylsulphathiazole solution

Phthalylsulphathiazole (20 mg) was weighed accurately and dissolved in hydrochloric acid (5 ml). The final volume was adjusted to 100 ml with
0.1 M hydrochloric acid.

2.2.3 Procedure

2.2.3.1 Sulphamethoxypyridazine

2.2.3.1.1 Determination of wavelength of maximum absorbance

Standard sulphamethoxypyridazine solution (2.0 ml) was pipetted into 10 ml volumetric flask. The MBTH solution (2.0 ml) was added to it and kept for 10 min at room temperature (30 degree). The ferric chloride solution (3.0 ml) was added to the reaction mixture and allowed to stand for 20 min at room temperature. The volume was adjusted to mark with water. The absorbance of the colored solution was scanned on Beckman model 25 spectrophotometer in the range of 500 to 600 nm against reagent blank.

Maximum absorbance was obtained at 565 nm (Fig.1).

Phthalylsulphathiazole, sulphamethoxazole, sulphaguanidine and sulphamerazine were treated similarly. The maximum absorbance was obtained at 565 nm with all the drugs.

However, Sulphaphenazole when treated in the similar manner, maximum absorbance was observed at 525 nm.
2.2.3.1.2 Lambert-Beer's curve for Sulphamethoxypyridazine

Standard sulphamethoxypyridazine solution (0.25, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 ml) was pipetted into a series of 10 ml volumetric flasks and analysed as described under 2.2.3.1.1. The absorbance of the reaction mixture was measured at 565 nm against reagent blank (Fig.2).

The Lambert-Beer's concentration range for sulphamethoxazole, sulphamerazine, sulphaguanidine sulphaphenazole and phthalylsulphathiazole are given in Table II. The absorbance of sulphaphenazole was measured at 525 nm.

2.2.4 Factors affecting the development of color

2.2.4.1 Effect of concentration of MBTH

Standard sulphamethoxypyridazine solution (2.0 ml) was treated with different volume of MBTH reagent solution and kept for 10 min at room temperature (30 degree). The reaction mixture was treated as described under 2.2.3.1.1. The absorbance of the reaction mixture was measured at 565 nm against reagent blank.

Maximum absorbance was observed in presence of 2.0 ml of MBTH reagent solution which remained constant on further increase in volume of MBTH.
2.2.4.2 Time for reaction with MBTH

Standard sulphamethoxypyridazine solution (2.0 ml) was treated with MBTH solution (2.0 ml). The reaction mixture was kept for different time interval at room temperature and analysed as described under 2.2.3.1.1.

The maximum color intensity was obtained after 10 min which remains constant for more than 20 min (Fig. 4).

2.2.4.3 Effect of concentration of Ferric chloride solution

Standard sulphamethoxypyridazine solution (2.0 ml) was treated with MBTH solution (2.0 ml) for 10 min at room temperature followed by addition of different volume of ferric chloride solution and analysed as described under 2.2.3.1.1.

Maximum color intensity was obtained in the presence of 2.0 ml of ferric chloride solution which remained constant with further increase in the volume of ferric chloride solution (Fig. 5).

In the present work, we have used 3.0 ml of ferric chloride solution.
2.2.4.4 Time for maximum color development and color stability

Standard sulphamethoxypyridazine solution (2.0 ml) was pipetted into a series of 10 ml volumetric flasks. MBTH solution (2.0 ml) was added to each flask and was allowed to stand for 10 min at room temperature. The ferric chloride solution (3.0 ml) was added to each flask and mixed thoroughly. It was allowed to stand for 5, 10, 15, 20, 30, 40 and 60 min at room temperature and analysed as described under 2.2.3.1.1.

Maximum absorbance was obtained after 20 min which remained constant for more than 2 hours (Fig.6).

Factors 2.2.4.1 to 2.2.4.4 were studied for remaining sulpha drugs. In case of sulphaphenazole the color of the reaction mixture was measured at 525 nm in the above experiments.

2.2.5 Analysis of Sulphonamides

2.2.5.1 Analysis of Sulphamethoxazole

Sulphamethoxazole (ca.20 mg) was weighed accurately and dissolved in and diluted to 100 ml with 0.1 M hydrochloric acid. The solution (2.0 ml) was analysed as described under 2.2.3.1.1.
The amount of sulphamethoxazole was determined by referring to the standard curve (Table III).

2.2.5.2 Analysis of Phthalylsulphathiazole

Phthalylsulphathiazole (ca. 20 mg) was weighed accurately and dissolved in hydrochloric acid (5 ml). The final volume was adjusted to 100 ml with 0.1 M hydrochloric acid. The solution (2.0 ml) was analysed as described under 2.2.3.1.1.

The amount of phthalylsulphathiazole was determined by referring to the standard curve (Table III).

2.2.5.3 Analysis of Sulphaguanidine, Sulphamerazine, Sulphaphenazole or Sulphamethoxypyridazine

Sulphaguanidine, sulphamerazine, sulphaphenazole or sulphamethoxypyridazine (ca. 40 mg) was weighed accurately and dissolved in and diluted to 100 ml with 0.1 M hydrochloric acid. An aliquot (10 ml) was diluted further to 50 ml with the same solvent. The resulting solution (2.0 ml) was analysed as described under 2.2.3.1.1.

For the analysis of sulphaphenazole the color was measured at 525 nm.

The amount of sulphaguanidine, sulphamerazine, sulphaphenazole or sulphamethoxypyridazine
was determined by referring to the standard curve (Table III).

2.2.5.4 Analysis of Sulphamethoxazole in combination with Trimethoprim in synthetic mixtures

Synthetic mixtures containing sulphamethoxazole and trimethoprim were prepared in laboratory (Table IV). The mixture equivalent to ca. 20 mg of sulphamethoxazole was weighed accurately and mixed with hydrochloric acid (20 ml, 0.1 M) and shaken thoroughly. The reaction mixture was filtered through Whatman No. 40 filter paper and the residue was washed thoroughly with 0.1 M hydrochloric acid. The filtrate and washings were combined in a 100 ml volumetric flask and diluted to the mark with 0.1 M hydrochloric acid. The resulting solution (2.0 ml) was analysed as described under 2.2.5.1 (Table IV).

2.2.5.5 Analysis of Sulphamethoxazole in combination with Trimethoprim in tablets

Twenty tablets were weighed and powdered. The powder equivalent to ca. 20 mg sulphamethoxazole was weighed accurately and analysed as described under 2.2.5.4 (Table V).
2.2.5.6 Analysis of Sulphamethoxazole tablets

Twenty tablets were weighed and powdered and analysed as described under 2.2.5.5 (Table V).

2.2.5.7 Analysis of Phthalylsulphathiazole tablets

Twenty tablets were weighed and powdered. The powder equivalent to ca. 20 mg phthalylsulphathiazole was weighed accurately and mixed with hydrochloric acid (5 ml) and shaken thoroughly. The reaction mixture was filtered through Whatman No. 40 filter paper and the residue was washed thoroughly with 0.1 M hydrochloric acid. The filtrate and washings were combined in a 100 ml volumetric flask and diluted to the mark with 0.1 M hydrochloric acid. The resulting solution (2.0 ml) was analysed as described under 2.2.5.2 (Table V).

2.2.5.8 Analysis of Sulphaguanidine, Sulphamerazine, Sulphaphenazole or Sulphamethoxypyridazine tablets

Twenty tablets were weighed and powdered. The powder equivalent to ca. 40 mg of sulphaguanidine, sulphamerazine, sulphaphenazole or sulphamethoxypyridazine was weighed accurately and mixed with hydrochloric acid (20 ml, 0.1 M) and shaken thoroughly. The reaction mixture was filtered through Whatman No. 40 filter paper and the
residue was washed thoroughly with 0.1 M hydrochloric acid. The filtrate and washings were combined in a 100 ml volumetric flask and diluted to the mark with 0.1 M hydrochloric acid. The resulting solution (2.0 ml) was analysed as described under 2.2.5.3 (Table V).

In case of sulphaphenazole color was measured at 525 nm.
2.3 RESULTS AND DISCUSSION

Various colorimetric procedures are reported for the estimation of sulphonamides. Most of them involve diazotisation of primary aromatic amino group by reaction with nitrous acid, prior to the coupling reaction. Several coupling agents suggested are either amines or phenols such as anthranilic acid, p-aminosalicylic acid, p-chloroaniline, m-aminophenol, p-aminobenzoic acid, N-(1-naphthyl) ethylenediamine dihydrochloride, etc. The excess of nitrous acid should be removed before addition of the coupling reagent. For this purpose, addition of sulphamic acid is suggested. To obtain reproducible results stringent time requirements between addition of reagents and for measurement of color must be adhere.

The proposed coupling reagent, MBTH, does not require usual diazotisation prior to coupling reaction. This avoids addition of chemicals such as sodium nitrite or sulphamic acid in the reaction mixture.

The mechanism of reaction of MBTH with aromatic amines is well-known. For sulphonamides it
can be proposed as follows:

\[
\begin{align*}
\text{II} + \text{Z} &= \text{SO}_2\text{NHR} \\
&= \begin{array}{c}
\text{CH}_3 \\
\text{N} = \text{N} \\
\text{Z} \\
\text{NHR}
\end{array}
\end{align*}
\]

The proposed method is based on the oxidative coupling of sulphonamides with MBTH to yield highly colored condensation products.

In proposed procedure, the solution of sulpha drug was reacted with MBTH in presence of ferric chloride. Various parameters of reaction such as MBTH, ferric chloride, and sulphonamides concentration, time for reaction and color development were studied to obtain maximum color intensity (Fig. 3-6).

The solution of sulpha drug on treatment with MBTH solution (2.0 ml) and ferric chloride solution (3.0 ml) gives highly colored condensation products. Maximum color intensity was obtained after
keeping the reaction mixture for 20 min at room temperature. The color intensity remains constant for more than 2 hours. Under the reaction conditions, colored products of sulphamethoxypyridazine, sulphaguanidine, sulphamerazine, sulphamethoxazole and phthalylsulphathiazole show maximum absorbance at 565 nm, while of sulphaphenazole show maximum absorbance at 525 nm. The Lambert-Beer's concentration range, molar absorptivity, Sandell's sensitivity and standard deviation for each sulphonamide are given in Table II and Table III.

The slopes, intercepts and correlation coefficients were obtained by linear regression analysis of the data (Table II). Pure samples of sulphamethoxypyridazine, sulphaguanidine, sulphamerazine, sulphamethoxazole, sulphaphenazole, phthalylsulphathiazole and their pharmaceutical dosage forms are analysed by proposed procedure. The results are in good agreement with those obtained by pharmacopoeial method (Table III & V). The proposed procedure was applied to assay sulphamethoxazole in combination with trimethoprim in synthetic mixtures (Table IV) as well as in tablets (Table V). The presence of trimethoprim as well as usual diluents and lubricants employed
in the formulation of dosage forms do not interfere in the proposed method.

The results and proposed mechanism of coupling reaction with MBTH indicate that free primary aromatic amino group is not involved in the coupling reaction. In coupling reaction with other agents the presence of primary aromatic amino group is essential. This is supported by the fact that the analysis of phthalylsulphathiazole can be carried out using this method without prior hydrolysis. Surprisingly, earlier it is reported that in MBTH procedure, phthalylsulphathiazole do not interfere in the determination.

The method is simple, precise and accurate.
Figure 1: Visible spectrum of the colored products obtained on reacting Sulphamethoxypyridazine with MBTH regent.
Figure 2: Lambert-Beer's curve for Sulphamethoxypyridazine.
Figure 3: Effect of concentration of MBTH solution.
Figure 4: Effect of time for reaction with MBTH.
Figure 5: Effect of concentration of Ferric chloride solution.
Figure 6: Effect of time for maximum color development and stability.
<table>
<thead>
<tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>mcg/ml</td>
<td>mcg/cm/0.001Å</td>
<td></td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Sulphamethoxy pyridazine</td>
<td>2-24</td>
<td>8.15</td>
<td>0.0344</td>
<td>0.2838</td>
<td>0.0056 0.9995</td>
</tr>
<tr>
<td>2</td>
<td>Sulphamerazine</td>
<td>2-24</td>
<td>6.95</td>
<td>0.0380</td>
<td>0.2612</td>
<td>0.0011 0.9998</td>
</tr>
<tr>
<td>3</td>
<td>Sulphaguanidine</td>
<td>2-16</td>
<td>13.30</td>
<td>0.0174</td>
<td>0.5704</td>
<td>-0.0078 0.9997</td>
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<tr>
<td>4</td>
<td>Phthalyl sulphathiazole</td>
<td>5-50</td>
<td>5.90</td>
<td>0.0683</td>
<td>0.1507</td>
<td>-0.0069 0.9999</td>
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<td>5</td>
<td>Sulpha methoxazole</td>
<td>5-70</td>
<td>2.96</td>
<td>0.0055</td>
<td>0.1161</td>
<td>0.0014 1.0000</td>
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<tr>
<td>6</td>
<td>Sulphaphenazole</td>
<td>1-10</td>
<td>16.40</td>
<td>0.0191</td>
<td>0.5224</td>
<td>-0.0002 0.9999</td>
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<td>Sr. No.</td>
<td>Name of drug</td>
<td>% recovered ± SD by Proposed Method</td>
<td>% recovered ± SD by Pharmacopoeial method</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>--------</td>
<td>---------------------------</td>
<td>-------------------------------------</td>
<td>-----------------------------------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Sulphamethoxypyridazine</td>
<td>99.33 ± 0.60</td>
<td>99.98 ± 0.63</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Sulphamerazine</td>
<td>99.98 ± 0.68</td>
<td>100.12 ± 0.54</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Sulphaguanidine</td>
<td>100.00 ± 0.73</td>
<td>99.91 ± 0.70</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>4</td>
<td>Phthalyl-sulphathiazole</td>
<td>98.99 ± 0.67</td>
<td>99.12 ± 0.65</td>
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<td></td>
<td></td>
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<tr>
<td>5</td>
<td>Sulphamethoxazole</td>
<td>100.78 ± 0.58</td>
<td>99.98 ± 0.61</td>
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<td></td>
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<tr>
<td>6</td>
<td>Sulphaphenazole</td>
<td>99.88 ± 0.69</td>
<td>100.32 ± 0.72</td>
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a: Standard deviation was calculated from the results of nine determinations.
### TABLE IV

**ANALYSIS OF SULPHAMETHOXAZOLE WITH TRIMETHOPRIM IN SYNTHETIC MIXTURE**

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Trimethoprim (in mg)</th>
<th>Sulphamethoxazole (in mg)</th>
<th>% recovered by Proposed method</th>
<th>% recovered by Pharmacopoeial method</th>
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<tbody>
<tr>
<td>1.</td>
<td>60</td>
<td>400</td>
<td>99.90</td>
<td>99.70</td>
</tr>
<tr>
<td>2.</td>
<td>80</td>
<td>300</td>
<td>99.85</td>
<td>99.90</td>
</tr>
<tr>
<td>3.</td>
<td>80</td>
<td>400</td>
<td>100.10</td>
<td>99.95</td>
</tr>
<tr>
<td>4.</td>
<td>160</td>
<td>800</td>
<td>99.84</td>
<td>99.80</td>
</tr>
<tr>
<td>5.</td>
<td>160</td>
<td>1000</td>
<td>99.98</td>
<td>99.95</td>
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</table>

*a*: Average value of five determinations.
### TABLE V

**ANALYSIS OF SULPHA DRUGS AND THEIR COMBINATIONS IN 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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<td>1.</td>
<td>POWDER</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>i)</td>
<td>Sulphamethoxypyridazine</td>
<td>99.55</td>
<td>99.62</td>
<td></td>
</tr>
<tr>
<td>ii)</td>
<td>Sulphamerazine</td>
<td>98.98</td>
<td>98.78</td>
<td></td>
</tr>
<tr>
<td>iii)</td>
<td>Sulphaguanidine</td>
<td>99.65</td>
<td>99.75</td>
<td></td>
</tr>
<tr>
<td>iv)</td>
<td>Phthalylsulphathiazole</td>
<td>93.99</td>
<td>98.87</td>
<td></td>
</tr>
<tr>
<td>v)</td>
<td>Sulphamethoxazole</td>
<td>100.10</td>
<td>100.15</td>
<td></td>
</tr>
<tr>
<td>vi)</td>
<td>Sulphaphenazole</td>
<td>99.75</td>
<td>99.78</td>
<td></td>
</tr>
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<td>2.</td>
<td>TABLETS</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>mg/Tab.</td>
<td>mg.</td>
<td>mg.</td>
<td></td>
</tr>
<tr>
<td>i)</td>
<td>Sulphamethoxypyridazine</td>
<td>500</td>
<td>501.60</td>
<td>508.40</td>
</tr>
<tr>
<td>ii)</td>
<td>Sulphamerazine</td>
<td>500</td>
<td>499.75</td>
<td>498.50</td>
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<tr>
<td>iii)</td>
<td>Sulphaguanidine</td>
<td>500</td>
<td>498.60</td>
<td>498.75</td>
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<td>iv)</td>
<td>Phthalylsulphathiazole</td>
<td>500</td>
<td>499.65</td>
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<td>v)</td>
<td>Sulphamethoxazole</td>
<td>500</td>
<td>498.84</td>
<td>499.20</td>
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<td>vi)</td>
<td>Sulphaphenazole</td>
<td>500</td>
<td>502.65</td>
<td>501.50</td>
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<td>vii)</td>
<td>Sulphamethoxazole</td>
<td>400</td>
<td>365.00</td>
<td>384.30</td>
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<td></td>
<td>Trimethoprim</td>
<td>80</td>
<td></td>
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<tr>
<td>viii)</td>
<td>Sulphamethoxazole</td>
<td>800</td>
<td>795.35</td>
<td>796.67</td>
</tr>
<tr>
<td></td>
<td>Trimethoprim</td>
<td>160</td>
<td></td>
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</table>

*a*: Average value of five determinations.