# Chapter III

## Materials and Methods

### Apparatus and Chemicals

1. Spectrophotometer - Pye Unicam SP 1800
2. Temperature controlled - water bath - Memmert
3. Piperazine citrate B.P. (100.3 % purity on dried basis as determined by official method[18])
4. Sulfonphthalein dyes
   - 4.1 Bromcresol green (BCG) - Fluka A.G.
   - 4.2 Bromthymol blue (BTB) - E. Merck
   - 4.3 Bromcresol purple (BCP) - Fluka A.G.
   - 4.4 Bromphenol blue (BPB) - B.D.H.
5. Chloroform - B.D.H.
6. Sodium hydroxide - Baker analyzed
7. Granular anhydrous sodium sulfate-Mallinckrodt
8. Picric acid-C.T. company
9. Ethanol, absolute-Reidel
10. Sulfuric acid-B.D.H.
11. Hydrochloric acid-Riedel

All of the chemicals were analytical grade obtained from various manufacturers. Piperazine citrate was pharmaceutical grade.

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Methods

1. Determination of wavelength for maximum absorption of complex between piperazine and four sulfonphthalein dyes

Reagents :

1.1 Dyes $4.00 \times 10^{-4}$ M were prepared as followed :

1.1.1 $4.00 \times 10^{-4}$ M BCG - BCG powder 69.8 mg. was dissolved in 250 ml. chloroform.

1.1.2 $4.00 \times 10^{-4}$ M BTB - BTB powder 62.4 mg. was dissolved in 250 ml. chloroform.

1.1.3 $4.00 \times 10^{-4}$ M BCP - BCP powder 54.0 mg. was dissolved in 250 ml. chloroform.

1.1.4 $4.00 \times 10^{-4}$ M BPB - BPB powder 67.0 mg. was dissolved in 250 ml. chloroform.

1.2 10 N Sodium hydroxide - Sodium hydroxide 90 gm. was dissolved in 190 ml. distilled water.

1.3 Chloroform

1.4 Granular anhydrous sodium sulfate

Standard Solution :

Piperazine citrate B.P. 0.3058 gm. (equivalent to 0.2432 gm. of piperazine hexahydrate) was accurately weighed into a 50-ml. volumetric flask and was made up to volume with

distilled water and mixed well to produce a concentration of 6.116 mg/ml .

General Procedure :

Ten ml. aliquot of standard solution was pipetted into 125-ml. separatory funnel, containing 25.0 ml. 10 N sodium hydroxide. Twenty five ml. chloroform was added and the solution was shaken vigorously for 1 minute and allowed to equilibrate for 5 minutes. The chloroform layer was drained off through a small piece of cotton wool surmounted by 1 gm. of granular anhydrous sodium sulfate (prewetted with about 2 ml. chloroform) into a 100-ml. volumetric flask. The stopper and tip of the separatory funnel were rinsed with chloroform. The extraction was repeated with 25.0 ml. and three further 10 ml. portions of chloroform, shaking vigorously 1 minute each time, draining chloroform through granular anhydrous sodium sulfate into the same volumetric flask. The funnel was rinsed down with chloroform and made to volume, mixed well (solution A).

Stock solution of piperazine was prepared by pipeting 10.0 ml. solution A into a 250-ml. volumetric flask and diluted with chloroform to volume. In a four 25-ml. volumetric flasks, 5.0 ml. of stock solution of piperazine and 5.0 ml. of $4.00 \times 10^{-4}$M of each dye were mixed and sufficient chloroform were added to volume. Reagent blank of each dye was prepared by pipetting 5.0 ml. of

$4 \times 10^{-4}$ M dye into 25-ml. volumetric flask, then chloroform was added to volume. The maximum absorption of the complex formed of each dye was determined spectrophotometrically by scanning in a 1-cm. cell against reagent blank under visible range from 360 to 680 nm.

The procedure was repeated five times with the same concentration of piperazine citrate. The result was a mean value of five determinations and the absorption spectra were shown in Figure 1. The reagent blank was used in all experiments because it was found no significant difference in absorbance between the reagent blank and the blank treated in the same procedure as described above.

2. Effect of Various Concentrations of Sodium Hydroxide on Releasing Piperazine Free Base from Piperazine Citrate

Reagents :

2.1 $4.00 \times 10^{-4}$M BCG - BCG 69.8 mg. was dissolved in 250 ml. chloroform.

2.2 Sodium hydroxide solution of various strengths were prepared as followed :

2.2.1 6 N Sodium hydroxide - Sodium hydroxide 54 gm. was dissolved in 190 ml. distilled water.

2.2.2 8 N Sodium hydroxide - Sodium hydroxide 72 gm. was dissolved in 190 ml. distilled water.

2.2.3 9 N Sodium hydroxide - Sodium hydroxide 81 gm. was dissolved in 190 ml. distilled water.

2.2.4 10 N Sodium hydroxide - Sodium hydroxide 90 gm. was dissolved in 190 ml. distilled water.

2.3 Chloroform

2.4 Granular anhydrous sodium sulfate

Standard Solution

Piperazine citrate B.P. 0.3058 gm.(equivalent to 0.2432 gm. of piperazine hexahydrate) was accurately weighed into a 50-ml. volumetric flask and was made up to volume with distilled water and mixed well to produce a concentration of 6.116 mg/ml.

General Procedure :

Ten ml. aliquot of standard solution was pipetted into four 125-ml. separatory funnels, each contained 25.0 ml. sodium hydroxide solution 6,8,9,10 normal respectively. Twenty five ml. chloroform was added and the solution was shaken vigorously for 1 minute, and allowed to equilibrate for 5 minutes. The chloroform layer was drained off through a small piece of cotton wool surmounted by 1 gm. of granular anhydrous sodium sulfate (prewetted with about 2 ml. chloroform). The filtrate was collected into a 100-ml. volumetric flask. The extraction with 25.0 ml. and two further 10 ml. portions of chloroform was repeated, shaking 1 minute each time. The funnel was rinsed down with chloroform, then changed the new volumetric flask. The aqueous phase was further extracted with 10.0 ml. chloroform (Extract No. 5), the filtrate was collected in a 25-ml. volumetric flask. The same procedure was proceeded with 10.0 ml. chloroform and collected the filtrate in another 25-ml. volumetric flask (Extract No. 6).

Two ml. of the Extract No. 5 and No. 6 were pipetted into two 25-ml. volumetric flasks, each containing 5 ml. of $4.00 \times 10^{-4}$ M BCG solution, mixing, the absorbance were measured against reagent blank in 1-cm. cell at wavelength of maximum absorption at 420 nm.

The procedure was repeated five times with the equal weights of piperazine citrate used. The mean values of five determinations were shown in Table 2 .

3. Preparation of Calibration Curve and Determination of Conformity to Beer's Law

Reagents :

3.1 Dyes $2.00 \times 10^{-3}$ M were prepared as follow :

3.1.1 $2.00 \times 10^{-3}$M BCG - BCG powder 0.3491 gm. was dissolved in 250 ml. chloroform.

3.1.2 $2.00 \times 10^{-3}$M BTB - BTB powder 0.3122 gm, was dissolved in 250 ml. chloroform.

3.1.3 $2.00 \times 10^{-3}$M BCP - BCP powder 0.2702 gm. was dissolved in 250 ml. chloroform.

3.1.4 $2.00 \times 10^{-3}$M BPB - BPB powder 0.3344 gm. was dissolved in 250 ml. chloroform.

Solution of lower dye concentration was prepared as followed :

3.1.5 Dye $1.00 \times 10^{-3}$ M was prepared by diluting 125.0 ml. $2.00 \times 10^{-3}$ M solution with chloroform to 250 ml.

3.1.6 Dye $4.00 \times 10^{-4}$ M was prepared by diluting 50.0 ml. $2.00 \times 10^{-3}$M solution of each dye with chloroform to 250 ml.

3.2 10 N Sodium hydroxide

3.3 Chloroform

3.4 Granular anhydrous sodium sulfate

Standard Solution :

Piperazine citrate B.P. 0.3058 gm. was accurately weighed into a 50-ml. volumetric flask and was made up to volume with distilled water and mixed well.

General Procedure :

Stock solution was prepared, using the same procedure as described under determination of wavelength for maximum absorption .

Five ml. of $1.00 \times 10^{-3}$ M BCG solution was pipetted into each of twelve 25-ml. volumetric flasks. One of these was diluted to volume with chloroform and used as a reagent blank. Stock solution of piperazine as hexahydrate 1.0, 2.0, 3.0, 4.0, 6.0, 8.0, 10.0, 12.0, 14.0, 16.0 and 18.0 ml. were pipetted into eleven of the volumetric flasks and were diluted to volume with chloroform, and mixed well. The absorbance of each concentration was measured versus reagent blank in 1-cm. cell. at 420 nm. With the same procedure; BTB, BCP and BPB were used as the color forming agent instead of BCG. The absorbance obtained were measured at wavelength of maximum absorption of 420 nm. for BTB, 410 nm. for BCP and 412 nm. for BPB.

The procedure was repeated four times with the equal weights of piperazine citrate used. The absorbance reading were used to prepare a calibration curve of each dye and to test for conformity to Beer's Law by plotting absorbance against

concentrations of piperazine as hexahydrate. The results were shown in Table 3 and Figure 2.

4. Effect of Dye Concentration on Absorbance of Piperazine -Dye Complex

Reagents :

4.1 Each dyes; BCG, BTB, BCP and BTB $4.00 \times 10^{-4}$M was prepared from stock solution under described for preparation of calibration curve.

4.2 10 N Sodium hydroxide

4.3 Chloroform

4.4 Granular anhydrous sodium sulfate

Standard Solution :

The same standard solution of piperazine citrate prepared under described for preparation of calibration curve was used.

General Procedure :

The same stock solution of piperazine as hexahydrate prepared under described for preparation of calibration curve was used. Various quantities of $4.00 \times 10^{-4}$M BCG : 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0 ml. were pipetted into eight separate 25-ml. volumetric flasks. To each of these, 4.0 ml. stock solution of piperazine as hexahydrate was added and made up to volume with chloroform. Reagent blanks were prepared by using various quantities of BCG as described above. The absorbances of the complex obtained were measured against reagent blanks.

This procedure was performed with BTB, BCP and BPB at wavelength of maximum absorption of each dye. The procedure was repeated four times with the aqual weights of piperazine citrate. Curve was plotted between milliliters of $4.00 \times 10^{-4}$M dye and absorbances. The results were shown in Table 4 and Figure 3. The mole ratios of piperazine as hexahydrate to each sulfonphthalein dye were calculated. Curve was plotted between mole ratios of dye to drug and absorbances. The results were shown in Table 4 and Figure 4.

5. Effect of Time on Stability of Piperazine-Dye Complex

Reagents :

5.1 Each dye; BCG, BTB, BCP and BPB $4.00 \times 10^{-4}$M was prepared from stock solution under described for preparation of calibration curve .

5.2 10 N Sodium hydroxide

5.3 Chloroform

5.4 Granular anhydrous sodium sulfate

Standard Solution :

The same standard solution of piperazine citrate prepared under described for preparation of calibration curve was used .

General Procedure :

The same stock solution of piperazine as hexahydrate prepared under described for preparation of cabration curve was used. Stock solution of piperazine as hexahydrate 6.0 ml. was pipetted into 50-ml. volumetric flask containing 10.0 ml. $4.00 \times 10^{-4}$M BCG and diluted to volume with chloroform, mixed well. Absorbance measurement was taken at time intervals 10, 20, 30, 40, 50, 60, 120, 180, 240, 300 and 360 minutes against reagent blank in 1-cm. cell at 420 nm.

BTB, BCP and BPB were also treated in the similar manner as described for BCG .

The procedure was repeated four times with the equal

weights of piperazine citrate used. Curves were constructed between time in minutes and absorbance. The results were shown in Table 5 and Figure 5.

6. Effect of Temperature on Stability of Piperazine-Dye Complex

Reagents :

6.1 Each dye; BCG, BTB, BCP, and BPB $4.00 \times 10^{-4}$ M was prepared from stock solution under described for preparation of calibration curve.

6.2 10 N Sodium hydroxide

6.3 Chloroform

6.4 Granular anhydrous sodium sulfate

Standard Solution :

The same standard solution of piperazine citrate prepared under described for preparation of calibration curve was used.

General Procedure :

The same stock solution of piperazine as hexahydrate prepared under described for preparation of calibration curve was used. Stock solution of piperazine as hexahydrate 3.0 ml. was pipetted into five 25-ml. volumetric flasks, each containing 5.0 ml. $4.00 \times 10^{-4}$ M BCG and 10.0 ml. chloroform. Four of them were immersed in temperature controlled-water bath for 15 minutes at temperatures of $40^{\circ}$, $50^{\circ}$, $60^{\circ}$, $70^{\circ}$, $\pm$ $1^{\circ}$C, respectively. Then, the solutions were cooled down to room temperature and diluted to volume with chloroform. Absorbances were determined at 420 nm. against reagent blank compared with the absorbance of piperazine-dye complex,

prepared at room temperature ($25^{\circ} \pm 1^{\circ}C$).

BTB, BCP and BPB were treated in the similar manner.

The procedure was repeated four times with equal weights of piperazine citrate. Curve were constructed between temperatures in degree. Celsius and absorbances. The results were shown in Table 6 and Figure 6.

Four sulfonphthalein dyes : BCG, BTB, BCP and BPB were compared statistically with each other in term of sensitivity of the complex formed, precision of determinations, amounts of the color forming agent used and stability of the complex formed to time and temperature The results were shown in Table 7. The most suitable dye was selected and used for quantitative determination of piperazine and its salts in various dosage forms of pharmaceutical preparations available in Thailand.

7. Determination of the Per Cent Labelled Amount of Piperazine as Hexahydrate in Piperazine Citrate Syrup USP XX Using Bromthymol Blue Method and Official Gravimetric Methods

Reagents :

7.1 Bromthymol Blue Method

7.1.1 $4.00 \times 10^{-4}$ M BTB in Chloroform

7.1.2 10 N Scdium hydroxide

7.1.3 Chloroform

7.1.4 Granular anhydrous sodium sulfate

7.2 Official Gravimetric Method

7.2.1 Trinitrophenol TS-The equivalent of 1 gm. of anhydrous trinitrophenol was dissolved in 100 ml. hot water, cooled the solution, and filtered if necessary.

7.2.2 Alcohol, absolute

Preparation of piperazine citrate syrup, USP XX

An anthelmintic syrup was compounded with the dose followed in piperazine citrate syrup USP XX (each 5 ml. of piperazine citrate syrup USP XX contained 500 mg. of piperazine as hexahydrate).

Ingredients per 500 ml.

| | |
|---|---|
| Piperazine citrate, BP | 62.88 gm. |

| | |
|---|---|
| Glycerin | 50 ml. |
| Peppermint spirit | 2.5 ml. |
| Propyl paraben | 10 mg. |
| Methyl paraben | 25 ml. |
| Green SJ. solution | |
| Syrup, USP | 250 ml. |
| Distilled water q.s. to | 500 ml. |

(The formula was modified from the formula used by ACDHON Co, Ltd.)

Piperazine citrate, BP 62.88 gm. (equivalent to 50 gm. of piperazine hexahydrate), glycerin, propyl paraben, methyl paraben were dissolved in about 250 ml. of syrup with the aid of gentle heat, cooled. Green SJ. solution and peppermint spirit were added and brought to 500 ml. with distilled water, filtered if necessary. To test the reproducibility of results and agreement between the methods, five replicate assays were performed using the bromthymol blue method and USP method[16].

7.1 Bromthymol Blue Method

The specific gravity of piperazine citrate syrup was determined and transferred an accurately weighed portion of the syrup equivalent to about 150 mg. of piperazine hexahydrate to a 50-ml. volumetric flask. The solution was diluted with distilled water to volume, mixed well. Ten ml. aliquot of solution was pipetted to 125-ml.

separatory funnel, containing 25.0 ml. 10 N sodium hydroxide. Twenty five ml. chloroform was added and the solution was shaken vigorously for 1 minute and allowed to equilibrate for 5 minutes. The chloroform layer was drained off through a small piece of cotton wool surmounted by 1 gm. of granular anhydrous sodium sulfate (prewetted with about 2 ml. chloroform) into a 100-ml. volumetric flask. The stopper and tip of the separatory funnel were rinsed with chloroform. The extraction was repeated with 25.0 ml. and three further 10 ml. portions of chloroform, shaking vigorously 1 minute each time, draining chloroform through granular anhydrous sodium sulfate into the same volumetric flask. The funnel was rinsed down with chloroform and made to volume, mixed well (solution A). Solution A 5.0 ml. was pipetted into 50-ml. volumetric flask and diluted to volume with chloroform and 2.0 ml. of aliquot solution was pipetted into a 25-ml. volumetric flask, containing 5.0 ml. $4 \times 10^{-4}$ M BTB, sufficient chloroform was added to volume. The absorbance of the yellow-complex formed was measured versus reagent blank in 1-cm. cell at 420 nm.

The exact amounts of piperazine as hexahydrate in syrup were determination from the calibration curve of piperazine BTB complex in Figure 7, using the following formula :

$$\% \text{ piperazine as hexahydrate} = \frac{Au \times Cs \times D \times S \times P}{As \times W \times L}$$

Au = Absorbance of the unknown

As = Absorbance of the standard

Cs = Concentration of the standard

D = Dilution factor

S = Specific gravity of syrup

L = Labelled amount

P = Per cent purity of piperazine citrate standard

W = Weight of Sample

To simplify the formula :

$$\% \text{ piperazine as hexahydrate} = \frac{Au \times S \times 33.87}{W \times L}$$

33.87 = Constant factor

7.2 Official Gravimetric Method

The official method[18] described for determination of piperazine citrate in piperazine citrate syrup USP XX was followed. The specific gravity of piperazine citrate syrup was determined. The amount of syrup equivalent to about 200 mg. of piperazine citrate was weighed in a 250-ml. beaker, 10 ml. of water and 75 ml. of trinitrophenol TS (picric acid) were added, stirred well, and allowed to stand in a refrigerator for not less than 2 hours. The precipitate was collected in a tared filtering crucible (British standard grade No. 4), washed with five 10 ml. portions of dehydrated alcohol and dried at 105°C to constant weight.

The percentage of piperazine as hexahydrate was calculated using the following formula :

$$\% \text{ piperazine as hexahydrate} = \frac{Wp \times S \times 35.68}{W \times L}$$

Wp = Weight of piperazine dipicrate formed

S = Specific gravity of syrup

W = Weight of sample

L = Labelled amount

35.68 = Constant factor

The results obtained from bromthymol blue method and official gravimetric method were given in Table 8 .

8. Determination of Percentage Recovery of Piperazine Citrate in Piperazine Citrate Syrup U.S.P. XX by Bromthymol Blue Method and Official Gravimetric Method[18]

The validity or accuracy of the bromthymol blue method was verified by adding various known amounts of standard piperazine citrate to piperazine citrate syrup U.S.P.XX which the exact amount of piperazine citrate was known. This syrup containing standard was tested and assayed for the amount of piperazine citrate according to the described method compared with the official gravimetric method.

8.1 Bromthymol Blue Method

The specific gravity of piperazine citrate syrup was determined and transferred an accurately weighed portion of the syrup, equivalent to about 50 mg. of piperazine hexahydrate into three 50 ml. volumetric flasks. The recoveries were determined by adding accurately weighed about 100, 125 and 150 mg. of standard piperazine citrate powder into the volumetric flasks, respectively and diluted to volume with distilled water. The same procedure was carried on under determination of the per cent labelled amount of piperazine as hexahydrate in piperazine citrate syrup U.S.P. XX. The procedure was repeated five times. The per cent recovery was calculated from per cent piperazine citrate found compared to per cent presented, per cent piperazine citrate in prepared syrup was corrected for

recovery as shown in Table 9.

$$\% \text{ recovery of piperazine citrate} = \frac{(Wf-Ws)}{Wa} \times 100$$

Wf = Weight of piperazine citrate found

Ws = Weight of piperazine citrate from syrup

Wa = Weight of piperazine citrate added

8.2 Official Gravimetric Method[18]

The specific gravity of piperazine citrate syrup was determined and transferred an accurately weighed portion of the syrup, equivalent to about 100 mg. of piperazine citrate to three 250 ml. beakers. Accurately weighed about 100, 125 and 150 mg. of standard piperazine citrate powder were added into the beakers, respectively. The rest of the procedure was the same as described under determination of the per cent labelled amount of piperazine citrate syrup U.S.P. XX, beginning with the word "10 ml. of water and 75 ml. of trinitrophenol TS were added, stirred well ----------". The procedure was repeated five times. The percentage recoveries were shown in Table 9

9. Analysis of Piperazine and Its Salts in Commercial Pharmaceutical Dosage Forms, Using Bromthymol Blue and Official Gravimetric Methods

Ten commercial preparations with various dosage forms containing piperazine and its salts were sampling from several manufacturers. The assay procedure by bromthymol blue method was compared with official gravimetric method. Name of various manufacturers and their pharmaceutical products were listed in Table 10.

Preparation of Samples

9.1 Syrups were divided into two categories

9.1.1 Piperazine citrate syrup USP

Each 5 ml. contains 500 mg. of piperazine as hexahydrate.

The general procedures for bromthymol blue method and official gravimetric method[18] were proceeded as in determination of per cent labelled amount of piperazine as hexahydrate in piperazine citrate syrup USP XX.

9.1.2 Piperazine citrate syrup BPC

Each 5 ml. contains 750 mg. of piperazine as hexahydrate

Bromthymol Blue Method

The same procedure described for determination of per cent labelled amount of piperazine citrate as hexahydrate in piperazine citrate syrup U.S.P. XX was followed.

Official Gravimetric Method(50)

Reagents :

9.1.2.1 Trinitrophenol solution. Sodium hydroxide solution (20.0 % W/V sodium hydroxide in water) 0.5 ml. was added to 100 ml. of saturated solution of trinitrophenol solution, freshly prepared.

9.1.2.2 1 N Sulfuric acid

9.1.2.3 Dehydrated alcohol

General Procedure :

Syrup about 1.5 gm. was accurately weighed and dissolved in a mixture of 3.5 ml. of 1 N sulfuric acid and 10 ml. of water; 100 ml. of trinitrophenol solution was added. The solution was heated on a water bath for 15 minutes, allowed to stand for 1 hour and filtered through a sintered-glass crucible (British Standard Grade No. 4). The residue was washed with

successive 10-ml. portions of a mixture of equal volumes of water and a saturated solution of trinitrophenol in water until the washings are free from sulfate, continued washing the residue with five sucessive 10-ml. portions of dehydrated alcohol, and the residue was dried to constant weight at 105°C.

The percent weight by volume was calculated as $C_{24}H_{46}N_6O_{14}$ by using the following formula.

$$\% \text{ W/V } C_{24}H_{46}N_6O_{14} = \frac{R}{W} \times S \times 39.35$$

$$\% \text{ piperazine as hexahydrate} = \frac{R \times S}{W \times L} \times 35.68$$

R = Grams of piperazine dipicrate formed

S = Specific gravity of syrup

W = Weight of syrup

L = Labelled amount

39.35 = Constant factor

35.68 = Constant factor

9.2 Elixir - Piperazine Citrate Elixir BPC 1980

Each 5 ml. contains 750 mg. of piperazine as hexahydrate.

The percentage of piperazine as hexahydrate in piperazine citrate elixir was determined by the methods, given for piperazine citrate BPC and bromthymol blue method.

9.3 Tablets - Piperazine Citrate Tablet USP XX

Plain and sugar coated tablets were assayed in the following way but in the case of sugar coated tablet, the sugar coated was washed out with distilled water and dried over silica gel before assayed.

Reagents :

The reagents as described under the determination of per cent labelled amount of piperazine as hexahydrate in piperazine citrate syrup USP XX was used.

General Procedures :

A total of 20 tablets were weighed and obtained an average weight per tablet, ground in a mortar without appreciable loss. In bromthymol blue method, an aliquot of the powder equivalent to about 150 mg. of piperazine as hexahydrate was weighed into a 50-ml. volumetric flask. Distilled water was added to volume and the solution was stirred magnetically for 1 hour, filtered through a dry filter paper(Whatman No. 1) discarding the first 20 ml. filtrate. Ten ml. of the filtrate

was transferred to 125-ml. separatory funnel which contained 25.0 ml. 10 N sodium hydroxide and proceeded as directed in the determination of per cent labelled amount of piperazine as hexahydrate in piperazine citrate syrup USP XX .

The percentage of piperazine as hexahydrate was calculated as followed :-

$$\% \text{ piperazine as hexahydrate} = \frac{Au \times A \times 33.87}{W \times L}$$

Au = Absorbance of the unknown

A = Average weight per tablet

W = Weight of powder taken

L = Labelled amount

33.87 = Constant factor

In the official method, the powder equivalent to about 200 mg. of piperazine citrate was weighed and transferred to a 250-ml. beaker. Ten ml. of a mixture of 1 part of dilute hydrochloric acid and 3 parts of water was added and stirred magnetically for 1 hour. The solution was filtered and the residue was washed with two 10-ml. portions of water. To the combined extracts and washings, 75 ml. of trinitrophenol TS was added and proceeded as directed in the assay under the determination of per cent labelled amount of piperazine as hexahydrate in piperazine citrate syrup USP XX, beginning with "stirred well, and allowed to stand in a refrigerator _ _ _ _".

The percentage of piperazine as hexahydrate was calculated as followed :-

$$\% \text{ piperazine as hexahydrate} = \frac{Wp \times A \times 35.68}{W \times L}$$

Wp = Weight of piperazine dipicrate formed

A = Average weight per tablet

W = Weight of sample

L = Labelled amount ; 35.68 = Constant factor

9.4 Capsule

A total of 20 capsules were weighed, each capsule was cut and squeezed paste content into a 250-ml. volumetric flask. The residue of paste in each capsule was washed with distilled water and combined the washing in 250-ml. volumetric flask. Distilled water was added to volume and stirred magnetically for 1 hour, filtered. The first 20 ml. filtrate was discarded. In bromthymol blue method, 10 ml. of this filtrate was transferred into a 125-ml. separatory funnel containing 25.0 ml. 10 N sodium hydroxide and proceeded as directed in determination of per cent labelled amount of piperazine as hexahydrate in piperazine citrate syrup USP XX. The 20 empty capsules were air-dried and weighed. The average of piperazine adipate in each capsule was obtained .

The percentage of piperazine adipate was calculaled as followed.: -

$$\% \text{ piperazine adipate} = \frac{Au \times C \times 2025.67}{W \times L}$$

Au = Absorbance of unknown

C = Average weight of piperazine adipate in each capsule

W = Weight of sample

L = Labelled amount of piperazine adipate per capsule

2025.67 = Constant factor

In official gravimetric method[52], 10.0 ml. aliquot of the filtrate was transferred to a 250-ml. beaker. Five ml. of dilute sulfuric acid and 50 ml. of trinitrophenol solution was added and the solution was heated on a waterbath for 15 minutes and allowed to stand for several hours. The solution was filtered through a sintered glass crucible and the residue was washed with successive quantities, each of 10 ml. of dehydrated alcohol and the residue was dried to constant weight at 105° C .

The percentage of piperazine adipate was calculated as followed :

$$\% \text{ piperazine adipate} = \frac{Wp \times A \times 42.68}{W \times L}$$

Wp = Weight of piperazine dipicrate formed

A = Average weight per capsule

W = Weight of sample

L = Labelled amount ; 42.68 = Constant factor

Five replicate assays were performed with each of commercial pharmaceutical preparation, both bromthymol blue method and official gravimetric methods[18,50,51]. The results were shown in Table 11.