CHAPTER III

MATERIALS AND METHODS

Instruments.

1. High pressure liquid chromatograph, Varian Aerograph 8500.

2. Column, Micro Pack Si-10, stainless steel, 8 mm i.d., 25 cm long.

3. Detector, Varichrom.

4. Recorder, Varian Aerograph 9176.

5. Syringe, 5 μl (Hamilton Company).

6. Potentiometer, Metrohm Herisau E 436.

7. Spectrophotometer, Hitachi 200-20 and recorder.

Chemicals.

1. Cimetidine, Lot 12-CIM. (99.76% purity on dried basis as determined by non-aqueous titration method)

2. Platinic chloride (BDH Chemical)

3. Potassium iodide (BDH Chemical)

4. Silica gel 60 G. (Merck)

5. Ammonia solution (Ajax chemical)

6. Chloroform (J.T. Baker Chemical)

7. Ethanol, absolute (BDH Chemical)

8. Ethyl acetate (Carlo Erba)

9. Glacial acetic acid (BDH Chemical)

10. Hexane (J.T. Baker Chemical)

11.Methanol   (Mallinckrodt)

12.Perchloric acid (Carlo Erba)

All chemical listed were analytical grade. Cimetidine was pharmaceutical grade.

Methods.

1. Selection of Solvent Composition Used as Mobile Phase for HPLC.

   1.1 Solubility test.

Reagents.

1. Chloroform:Methanol ratio 50:50, 60:40, 70:30 and 80:20

2. Ethyl acetate:Methanol 50:50, 60:40, 70:30 and 80:20

3. Hexane:Methanol 50:50, 60:40, 70:30 and 80:20

4. Hexane:Ethanol 50:50, 60:40, 70:30 and 80:20

Method.

Cimetidine standard 20 mg was accurately weighed into a 50 ml glass-stoppered centrifuge tube, 10.0 ml methanol was added, shake well and the solubility was observed.

The procedure was repeated by using ethanol, chloroform hexane, ethyl acetate, mixture of chloroform and methanol, hexane and methanol, hexane and ethanol and ethyl acetate and methanol in the ratio of 50:50, 60:40, 70:30 and 80:20, res-pectively, as solvents. The results obtained were shown in Table 1.

   1.2 Thin layer chromatography.

Reagents.

1. Chloroform:Methanol ratio 70:30, 80:20 85:15, and 90:10

2. Ethyl acetate:Methanol ratio 80:20

3. Hexane:Ethanol ratio 80:20

4. Chloroform:Methanol:Ammonia solution ratio 70:30:0.5, and 80:20:0.5

5. Iodoplatinate spray.

Platinic chloride 0.25 g and potassium iodide 5 g are dissolved in sufficient water to produce 100 ml (32).

Thin layer chromatographic plates.

Silica gel 60 G plates.

Standard preparation.

Cimetidine standard was dissolved in methanol to obtain a solution having a concentration of 10 mg/ml.

Sample preparation.

The sample was dissolved in methanol to obtain a solution having a concentration of 10 mg cimetidine/ml.

Method.

Ten μl (100 μg) of standard and sample solution were spotted 2 cm. from the lower edge of the silica gel plate. The spotted plate was placed in a chromatographic tank containing mixture of chloroform:methanol (80:20) which was equilibrated with the solvent vapour for 15 minutes. Plate was developed through a distance of 10 cm and was dried at room temperature in a current of air until solvent was no longer detectable. The spots were detected with iodoplatinate spray. The Rf values and shape of spots were noted.

The procedure was repeated by using mixture of chloro-

form:methanol (70:30, 85:15, and 90:10), ethyl acetate:metha-
nol (80:20), and hexane:ethanol (80:20) as developing solvents.
The sample in the injection dosage form, was developed in mix-
ture of chloroform:methanol:ammonia solution (70:30:0.5, and
80:20:0.5). The procedure was repeated three times and the re-
sults obtained were shown in table 2.

2. Selection of the Optimum Flow Rate, Pressure and Retention
   Time.

Chromatographic conditions.

    The liquid chromatograph was operated at ambient tem-
perature with UV detection at wavelength 240 nm, sensitivity
of 0.1 absorbance unit full scales (AUFS). The recorder was
adjusted at 1 cm/ml.

Column.

    Micro Pack column, Si-10.

Mobile phases.

    Two mobile phase solvents were used. Solvent I contained
70% of chloroform and 30% of methanol. Solvent II contained
80% of chloroform and 20% of methanol. Mobile phases were de-
gased prior to use.

Standard solution.

    Cimetidine 1 mg/ml in mobile phase.

Chromatographic procedure.

    Triplicate 5µl aliquots of standard solution was chro-
matographed by using flow rate at 1.5, 2, 2.5, and 3 ml/min

respectively. The pressure and retention time were observed. The results obtained were shown in Table 3 and Figure 2.

3. Determination of Maximum Absorption Wavelength.

3.1 Spectrophotometric method.

Cimetidine 50 mg was accurately weighed and dissolved in the mixture of chloroform:methanol (70:30) 50.0 ml. The solution was diluted to produce a concentration of 10 μg/ml. The maximum absorption wavelength of cimetidine was determined spectrophotometrically by scanning in a 1 cm cell in the ultra- violet range from 200-350 nm using mixture of chloroform: methanol (70:30) as blank. The absorption spectrum obtained was shown in Figure 3.

3.2 HPLC method.

Chromatographic conditions.

The liquid chromatograph was operated at ambient tem- perature with UV detection at wavelength 200-280 nm, sensiti- vity of 2 AUFS. The solvent flow rate was 2 ml/min and the recorder was adjusted at 1 cm/min.

Column.

Micro Pack column, Si-10.

Mobile phase.

Solvent I.

Standard solution.

Cimetidine 1.0 mg/ml in mobile phase.

Chromatographic procedure.

Duplicate 5 µl aliquots of standard solution were chromatographed. Each 5 wavelength was changed after stopping the column flow. The absorption spectrum obtained was shown in Figure 4.

4. Determination of Adherence to Beer's Law.

Chromatographic conditions.

The liquid chromatograph was operated as in the selection of the optimum flow rate, pressure and retention time. The solvent flow rate was 3 ml/min.

Mobile phase.

Solvent 1.

Chromatographic procedure.

Six solution of cimetidine were prepared to contain between 0.3 and 1.8 mg/ml in the mobile phase. Triplicate 5 µl aliquots of each solution were chromatographed. The calibration curve was plotted between peak heights (and peak areas) against the concentrations of cimetidine. The results obtained were shown in Table 4 and Figure 5 and 6.

5. Determination of the Reproducibility of Peak Area and
   Peak Height.

   Chromatographic conditions, column, and mobile phase
were described as under determination of adherence to Beer's
law.

Chromatographic procedure.

   Five μl aliquots of cimetidine solution, 1.5 mg/ml
were chromatographed ten times. The results obtained were
shown in Table 5.

6. Determination of the Percent Labelled Amount of Cimetidine
   in Cimetidine Tablet by Using HPLC Method, Non-aqueous Ti-
   tration Method and Spectrophotometric Method.

   6.1 HPLC method.

   Chromatographic conditions, column, and mobile phase
were described as under determination of adherence to Beer's
law.

Standard solution.

   Cimetidine 1.5 mg/ml in mobile phase.

Chromatographic procedure.

   Twenty cimetidine tablets (200 mg/tab.) were weighed
and finely powdered. An accurately weighed portion of the pow-
der equivalent to about 75 mg of cimetidine was transferred
to a 50 ml volumetric flask. Then about 30 ml of chloroform:
methanol (70:30) was added, shaken for 10 minutes and the

solvent was added to 50.0 ml, mixed well. The solution was filtered through Whatman filter paper No.1, the first 15 ml filtrate was discarded and the remained filtrate was collected. Triplicate 5 μl aliquots of filtrate were chromatographed. Each sample run was immediately followed by a run of the standard solution.

Calculations.

Since the identical volume of standard solution was injected after the sample solution was eluted, the percent of labelled amount found was calculated as follow:

$$\text{Percent of labelled amount} = \frac{H_p}{H_s} \times \frac{C_s}{C_p} \times 100$$

where, H_p = peak height of assay sample solution in mm.

H_s = peak height of standard solution in mm.

C_p = concentration of cimetidine in mg/ml of assay preparation.

C_s = concentration of cimetidine in mg/ml of standard solution.

6.2 Non-aqueous titration method.

Reagent.

0.1 N Perchloric acid.

8.5 ml of perchloric acid and 500 ml of glacial acetic acid were mixed. The solution was cooled, and glacial acetic acid was added to make 1000 ml.

Procedure.

Twenty cimetidine tablets (200 mg/tab) were weighed and finely powdered. A portion of the sample mixture equivalent to about 200 mg of cimetidine was accurately weighed and transfered into 100-ml beaker. Fifty ml of glacial acetic acid was added and stirred for 5 minutes and then titrated with 0.1 N perchloric acid. End point was determined potentiometrically by using combined glass electrode. The amount of cimetidine in tablet was calculated from the following formula:

Percent of labelled amount

$$= \frac{\text{ml titrant} \times \text{N. perchloric acid} \times 25.234 \times \text{wt/tab(g)} \times 100}{\text{sample wt(g)} \times 200}$$

where, 25.234 = milliequivalent of cimetidine.

6.3 Spectrophotometric method.

Reagent.

0.1 N Sulfuric acid.

Add slowly, with stirring, 3 ml of sulfuric acid to about 1020 ml of water, allow to cool to 25°C.

Procedure.

Twenty cimetidine tablets (200 mg/tab) were weighed and finely powdered. An accurately weighed portion of the powder, equivalent to about 100 mg of cimetidine, was transferred into 200 ml volumetric flask, about 100 ml of 0.1 N sulfuric acid was added and shaken for 15 minutes. The solution was diluted to volume with 0.1 N sulfuric acid, mixed thoroughly, and filtered with Whatman filter paper No.1, discarded the first 15 ml of filtrate. The filtrate was diluted to have a concentration about 10 μg/ml with 0.1 N sulfuric acid. The absorbance of sample and standard solution, concentration 10 μg/ml in 0.1 N sulfuric acid, were concomitantly determined in 1cm-cell at wavelength 218 and 260 nm against 0.1 N sulfuric acid as blank. The amount of cimetidine in tablet was calculated from the following formula:

$$\text{Percent labelled amount} = \frac{(A_{218}-A_{260})_p}{(A_{218}-A_{260})_s} \times \frac{C_s}{C_p} \times 100$$

where, $(A_{218}-A_{260})_p$ = the different absorption between 218 and 260 nm of sample.

$(A_{218}-A_{260})_s$ = the different absorption between 218
and 260 nm of standard.

C_p = concentration of cimetidine in μg/ml of sample
solution.

C_s = concentration of cimetidine in μg/ml of standard
solution.

All methods were repeated **ten** times and the results
obtained were shown in Table 6 and Figure 7, 8 and 9 .

7. Determination of the Percent Recovery of Cimetidine in
Cimetidine Tablet by HPLC Method, Non-aqueous Titration
Method and Spectrophotometric method.

7.1 HPLC method.

Chromatographic conditions, column and mobile phase
were described as under determination of adherence to Beer's
law.

Standard solution.

Cimetidine 1.5 mg/ml in mobile phase.

Chromatographic procedure.

Various amounts of cimetidine (2.0, 6.0 and 10.0
mg) were added to 4.0 ml of known amount of cimetidine (8 mg)
in the assay solution, and the solutions were diluted to 10.0
ml with mobile phase, mixed well. Triplicate 5μl aliquots of
each solution were chromatographed. Each sample run was imme-
diately followed by a run of the standard solution.

The total amount of cimetidine was calculated from the following formula:

$$\text{Total amount of cimetidine } (W_t) = \frac{H_p}{H_s} \times C_s \times 10$$

where, H_p = peak height of assay sample solution in mm.

H_s = peak height of standard solution in mm.

C_s = concentration of cimetidine in mg/ml of standard solution.

10 = dilution factor.

And then the percent recovery was calculated from the following formula:

$$\% \text{ recovery of cimetidine} = \frac{(W_t - W_s)}{W_a} \times 100$$

where, W_t = total amount of cimetidine found.

W_s = amount of cimetidine from assay solution.

W_a = amount of cimetidine added.

7.2 <u>Non-aqueous titration method</u>.

<u>Procedure</u>.

Several aliquots of the sample mixture (which the amount of cimetidine was known from the previous determination) equivalent to about 200 mg of cimetidine were accurately weighed and transfered to 100 ml beakers. Then 2.0 ml of cimetidine standard solution, 1, 3 and 5 mg/ml in absolute ethanol, were added respectively. Fifty ml of glacial acetic acid was added and stirred for five minutes and then titrated with 0.1 N perchloric acid. End point was determined potentiometrically by using combined glass electrode.

The total amount of cimetidine was calculated from the following formula:

Total amount of cimetidine

= ml titrant $\times$ N. perchloric acid $\times$ 25.234

And then the percent recovery was calculated as described under HPLC method.

7.3 Spectrophotometric method.

Procedure.

Several aliquots of the sample mixture (which the amount of cimetidine was known from the previous determination) equivalent to about 90 mg of cimetidine were accurately weighed and transfered to 200 ml volumetric flask. Then 2.0 ml of cimetidine standard solution, 1, 3 and 5 mg/ml in 0.1 N sulfuric acid, were added respectively. The rest of the procedure was the same as described under determination of the percent labelled amount of cimetidine in cimetidine tablet, beginning with the word " about 100 ml of 0.1 N sulfuric acid was added and shaken for 15 minutes. --- ".

The total amount of cimetidine was calculated from the following formula:

Total amount of cimetidine

$$= \frac{(A_{218}-A_{260})_p}{(A_{218}-A_{260})_s} \times C_s \times \text{dilution factor}$$

And then the percent recovery was calculated as described under HPLC method.

All methods were repeated three times and the results obtained were shown and compared in Table 7.

8. <u>Comparative Analysis of Pharmaceutical Preparations Contain-
ing Cimetidine</u>.

To test the validity of the method, eight commercially
available formulations with different dosage forms were analyzed
by HPLC method   compared with non-aqueous titration method
and spectrophotometric method.

8.1 <u>HPLC method</u>.

8.1.1 <u>Tablet and capsule</u>.

The same procedure was carried on as described under
determination of percent labelled amount of cimetidine in ci-
metidine tablet.

8.1.2 <u>Injection</u>.

Chromatographic conditions and column were described
under determination of adherence to Beer's law.

<u>Mobile phase</u>.

Mobile phase contained 75% of chloroform, 25% of
methanol and 0.5% ammonia solution and was degassed prior to
use.

<u>Standard solution</u>.

Cimetidine 1.0 μg/ml in mobile phase.

<u>Chromatographic procedure</u>.

An accurately measured volume of sample solution,
equivalent to about 100 mg of cimetidine was transfered to a
50 ml volumetric flask and mobile phase was added to 50.0 ml,
mixed well. Five ml quantities of solution was pipeted into

10 ml volumetric flask and diluted to volume with mobile phase.
Triplicate 5 μl aliquots of sample solution were chromatographed.
Each sample run was immediately followed by a run of the
standard solution. The percent labelled amount found was cal-
culated as described under the determination of the percent
labelled amount of cimetidine in cimetidine tablet. The pro-
cedure was repeated three times. The chromatogram was shown
in Figure 10.

8.2 Non aqueous titration method.

Tablet and capsule.

The same procedure was carried on as described under the
determination of the percent labelled amount of cimetidine in
cimetidine tablet.

8.3 Spectrophotometric method.

8.3.1 Tablet and capsule.

The same procedure was carried on as described under
the determination of the percent labelled amount of cimetidine
in cimetidine tablet.

8.3.2 Injection.

An accurately measured volume of sample solution,
equivalent to about 100 mg of cimetidine was transfered into
a 200 ml volumetric flask, 0.1 N sulfuric acid was added to
volume and mixed thoroughly. One ml of this solution was trans-
fered into 50 ml volumetric flask and diluted to volume with
0.1 N sulfuric acid, mixed. The absorbance of sample and

standard solution, concentration 10 μg/ml, were concomitantly determined in 1 cm cell at wavelength 218 and 260 nm against 0.1 N sulfuric acid as blank. The amount of cimetidine was calculated as described under the determination of the percent labelled amount of cimetidine in cimetidine tablet. The procedure was repeated three times. The absorption spectra were shown in Figure 11.

The results obtained from all methods were shown and compared in Table 9.