RESULTS AND DISCUSSION

Chromatographic conditions.

Adsorption chromatography or liquid solid chromatography was selected as a separation mode. This mode of chromatography has been most successful in separation of compounds with low molecular weight (<1000), low to moderate polarity, non ionic, differing in the number, type or position of their functional groups and good solubility in organic solvents.

Silica, totally porous, 10 μm diameter was used as the stationary phase. It is chemically stable, stable under high pressure. Moreover, it can be repeatedly without deterioration, and the distribution coefficient (K) are affected only to a small extent by temperature. There is no need for thermostatted columns and most adsorption chromatographic separation can be done at ambient temperature.

Selection of Solvent Composition Used as Mobile Phase for HPLC.

Solubility test.

The solubility of sample in mobile phase is an important criterion for selection of a good mobile phase. In addition, it should be pure, since the impurity will cause a higher base line offset, thus increasing the noise base line (33). The solvent strength should be considered in order to obtain a good

chromatographic resolution. The elution strength increases with increasing polarity. The mobile phase systems used in adsorption chromatography are most often binary mixtures of the organic solvents which are small differences in elution strength.

Cimetidine has a cyanoguanidine functional group which is weakly basic, has high dipole moment, hydrophilicity and hydrogen-bonding properties, therefore, cimetidine is a polar molecule. From the experimental data as shown in Table 1, cimetidine was soluble in ethanol and methanol which are polar solvents. In moderate and low polar solvents, such as chloroform, ethyl acetate and hexane, cimetidine was less soluble. In binary mixtures of organic solvents, cimetidine was soluble in mixture of chloroform:methanol, ethyl acetate:methanol in ratio of 50:50, 60:40, 70:30 and 80:20, respectively. Hexane and methanol were immiscible because of their large difference in polarity. Cimetidine was soluble in mixture of hexane and ethanol in ratio 50:50, 60:40 and 70:30, but in ratio 80:20, cimetidine was less soluble, since the solvent mixture was less polar.

Thin layer chromatography.

Thin layer chromatography is analogous to adsorption chromatography and extrapolations to adsorption chromatographic column can be made from thin layer chromatographic data. Stewart et al (34) compared high pressure liquid solid chromatography and found comparable separations. Any sample type that has been successfully chromatographed by thin layer chro-

matography can be separated by adsorption chromatography. R_f value was related to k' (capacity factor) as shown in Figure 1. The higher R_f value, the lower k'. If k' values are too low, the components elute too quickly. Therefore, the solvent is too strong. Conversly, if k' values are too high, the elution times are too long.

The binary mixtures of solvents were used as developing solvents for thin layer chromatography as shown in Table 2. In ratio 80:20, mixture of chloroform:methanol gave higher R_f value than mixture of ethyl acetate:methanol and hexane:ethanol. In mixture of chloroform:methanol, ratio 70:30 gave higher R_f value than ratio 80:20, 85:15, and 90:10. The mixtures of chloroform:methanol, ratio 70:30 and 80:20 which had R_f value 0.90 and 0.80 and k' value 0.11 and 0.25, respectively, were selected as mobile phases for selection of flow rate and retention time for the analysis of cimetidine.

Cimetidine injection contains cimetidine hydrochloride as active ingredient. The developing solvent was modified by adding ammonia solution to make alkali and cimetidine hydrochloride was converted to cimetidine base. Sample in injection dosage form and standard cimetidine were developed in mixture of chloroform:methanol:ammonia solution, ratio 70:30:0.5. R_f value of cimetidine was 0.95 and k' value was 0.05, k' value was too low. The solvent strength was decreased by decreasing ratio of methanol and increasing ratio of chloroform. The mixture of chloroform:methanol:ammonia solution, ratio 75:25:0.5

gave R_f value 0.82 and k' value 0.22. Therefore, this solvent was selected as mobile phase for the analysis of cimetidine hydrochloride.

Selection of Flow Rate and Retention Time.

       Cimetidine was chromatographed by using chloroform: methanol, ratio 70:30 and 80:20 as mobile phases with flow rate 1.5 ml/min. The peaks of cimetidine were detected at a retention time of 11.35 and 10.20 minutes, respectively. With flow rate 2.0, 2.5 and 3.0 ml/min, the peaks were detected at a retention time of 8.30 and 7.18, 6.04 and 5.00 and 4.28 and 3.10 minutes, respectively, as shown in Table 3. The long retention times were decreased by increasing the flow rate. Chromatograms obtained by using both mobile phases with flow rate 3.0 ml/min were shown in Figure 2. The mobile phase, ratio 70:30 gave better resolution chromatogram with shorter retention time and narrower peak than that obtained from mobile phase, ratio 80:20. Therefore, the mobile phase, chloroform: methanol, ratio 70:30 and flow rate 3 ml/min were selected for the analysis of cimetidine.

       Cimetidine hydrochloride was chromatographed by using chloroform:methanol:ammonia solution, ratio 75:25:0.5 as mobile phase with flow rate 3.0 ml/min and the peaks were detected at a retention time of 3.49 minutes.

Determination of Maximum Absorption Wavelength.

The absorption spectra or absorption maximum of the compound can be obtained by spectrophotometry and wavelength scanning of a peak after stopping the column flow during an HPLC run with variable wavelength UV-detector.

In the experiment, cimetidine was dissolved in mobile phase, chloroform:methanol ratio 70:30 and the solution was scanned spectrophotometrically in the ultraviolet range from 200-350 nm. The absorption spectrum showed the absorption peak at 235 nm as shown in Figure 3. In HPLC method, the absorption spectrum showed the maximum absorption in wavelength range 235-240 nm as shown in Figure 4. The wavelength 240 nm was used to operate the HPLC with other parameters and the chromatogram obtained had a good resolution.

Determination of Adherence to Beer's Law.

For quantitative HPLC analyses, linearity of detectors is one of the instrumental requirements. Since the peak height and peak area are proportional to the amount of compound eluted, then the relationship between peak height or peak area and concentration of compound should be adherence to Beer's law. For UV detectors, large band-width of spectral source and deviations from Beer's law can be the major source of non-linearlity.

A plot of peak height and peak area versus the amount of cimetidine injected was linear over the concentration range

of 1.5 - 9.0 µg per 5 µl, with correlation coefficients of 0.9991 and 0.9985, respectively. The curves extrapolated through the origin with a negligible intercept as shown in Figure 5.

Determination of the Reproducibility of Peak Area and Peak Height.

In the external standard method, absolute amounts are calculated from peak heights or peak areas found in the sample analysis and the corresponding response factors previously obtained from the standard solution. Since absolute amounts are measured, high precision of injection repeatability is required.

Data in Table 5 was generated by running 10 analyses. It showed the precision of peak height and peak area measurements with coefficients of variation of 0.47 % and 1.75 %, respectively. Since the precision of peak height measurement was better, therefore, it was used in calculation of the amount of cimetidine.

After studying the operating parameters for the chromatographic procedure, the optimum operating conditions were selected for quantitative determination of cimetidine in pharmaceutical preparations as follow:

Column	Micro Pack Si-10
Mobile phase	chloroform:methanol 70:30
Flow rate	3 ml/min (pressure 75 atm)
Sample size	5 μl (solution of cimetidine 1.5 mg/ml in mobile phase)
UV-detector	240 nm, 0.1 AUFS
Chart speed	1 cm/min

For the determination of cimetidine hydrochloride, mobile phase, chloroform:methanol:ammonia solution, ratio 75:25:0.5 was used.

Determination of the Percent Labelled Amount of Cimetidine in Cimetidine Tablet Using HPLC Method, Non-aqueous Titration Method and Spectrophotometric Method.

The content of cimetidine in cimetidine tablet was determined by HPLC method, non-aqueous titration method and spectrophotometric method. The results obtained were compared in Table 6 . The mean percentage value for ten determinations was 99.70 with 0.25 % of coefficient of variation by HPLC method, 100.24 with 0.74 % of coefficient of variation by non-aqueous

titration method and 98.68 with 0.37 % of coefficient of varia-
tion by spectrophotometric method. The data presented showed
a good precision and a close relationship between the three
methods. It was indicated that HPLC method gave reproducibility
results compare well with those obtained from non-aqueous ti-
tration method and spectrophotometric method.

The results obtained were within the general limit of
content in tablet (95.0- 105.0 %) thus this preparation was
continued using for the purpose of testing the accuracy of
the method.

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

The accuracy of the proposed method was checked by
determining the percentage recovery. Since other in-active
excipients in the preparation might interfere with the deter-
mination of sample. Therefore, these interferences could be
detected by adding standard cimetidine of various amounts into
cimetidine tablet, which the exact amount of cimetidine was
known and determined by using the proposed procedure. The mean
percent recoveries were calculated from triplicate determina-
tions (Table 7). For the weight of cimetidine added: 2, 6
and 10 mg, HPLC gave the percent recoveries of 99.78, 99.85
and 100.36 (mean value of percent recovery was 100.00 with
95 % confidence limits of $\pm$ 0.79) with 0.47, 0.73 and 0.60 %

of coefficients of variation, respectively. In non-aqueous
titration method, the percent recoveries were 99.19, 101.12,
and 100.99 (mean value of percent recovery was 100.17 with
95 % confidence limits of ± 2.38) with 0.05, 0.25 and 0.44 %
of coefficients of variation, respectively. In spectrophoto-
metric method, the percent recoveries were 99.46, 101.65 and
101.51 (mean value of percent recovery was 100.87 with 95 %
confidence limits of ± 3.5) with 1.23, 0.78 and 0.14 % of
coefficients of variation, respectively. The results showed
that all methods produced good recoveries with high reprodu-
cibility. Therefore, the presence of other excipients produced
no effect on the chromatographic procedure and UV-detection
of cimetidine determination.

Comparative Analysis of Pharmaceutical Preparations Containing Cimetidine.

To test the validity of the method, eight commercially
available formulations with different dosage forms were analyz-
ed by HPLC method compared with non-aqueous titration method
and spectrophotometric method. In HPLC method, cimetidine in
tablet and capsule were determined by using chloroform:methanol
ratio 70:30 as mobile phase. In injection dosage form, cimeti-
dine was protonated with hydrochloric acid to form cimetidine
hydrochloride and it was determined in the form of cimetidine
base by using chloroform:methanol:ammonia solution, ratio 75:
25:0.5 as mobile phase. Cimetidine in injection preparations

could not be determined by non-aqueous titration method because inactive substances, such as vehicles, interfered reaction of perchloric acid and cimetidine. Besides, cimetidine could not be extracted from aqueous alkali solution, since it is a weakly basic compound and have hydrophilicity and hydrogen bonding properties. Figure 12 showed the thin layer chromatogram of cimetidine residue which were extracted from aqueous alkali solution. The residue of the tenth extraction still showed the present of cimetidine.

Table 9 shows the results of mean value of triplicate determinations of each sample expressed in percentage of the amount labelled. In tablet, the results were in close value with 0.05 - 0.43 % of coefficients of variation for HPLC method, 0.04 - 0.32 % for titration method, and 0.11 - 0.34 % for spectrophotometric method. In capsule, the coefficients of variation were 0.22 -00.65 % for HPLC method, 0.36 - 0.38 % for titration method, and 0.40 - 0.56 for spectrophotometric method In injection, the coefficients of variation were 0.22 - 0.52 % for HPLC method and 0.26 - 0.38 % for spectrophotometric method. The results obtained indicated that the proposed method could be used for the determination of cimetidine and cimetidine hydrochloride in pharmaceutical preparations compared well with both non-aqueous titration and spectrophotometric method.

An internal standard could have been used in this study in order to minimize the error due to sample injection and column condition. However, this necessitates finding a chemically inert compound with suitable chemical and physical properties and a retention time which will not interfere with the compound under investigation. This is frequently a trial and error procedure and often results in a chromatogram which requires an extended analysis time. The use of an external standard requires that all operating parameters remain resemble those of standard. In high pressure liquid chromatography, variation may arise in mobile phase composition, flow rate and temperature. The sample was therefore chromatographed along with a standard to obtain the greatest precision and accuracy.