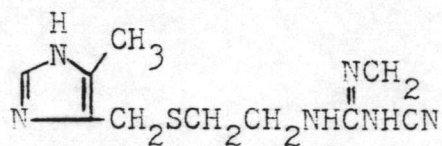INFORMATION

Chemistry.

Cimetidine [N"-Cyano-N-methyl-N'-[2[[-(5-methyl-1H-imidazole-4-yl)methyl]thio]ethyl]guanidine] has the following structure:

Cimetidine was synthesized by J.W.Black,et al(4) for the Smith Kline and French Company. It was obtained by two synthetic methods. Method I.A solution of 4-[(2-aminoethyl)thiomethyl]5-methylimidazole and N-cyano-N', S-dimethylisothiourea in acetonitrile was heated under reflux for 48 hrs.The product obtained was recrystallized from CH_3CN to yield cimetidine. Method II. 4-[(2-Aminoethyl)thiomethyl]-5-methylimidazole in ethanol was added slowly with stirring to a solution of dimethylcyanodithioimidocarbonate in ethanol at room temperature. The mixture was set aside overnight and filter to afford N-cyano-N'-[2-(5-methylimidazol-4-yl)methylthioethyl] - S-methylisothiourea in two crops. A solution of $MeNH_2$ in ethanol was added to a solution of the isothiourea in ethanol and the mixture was set aside at room temperature for 2.5 hrs. Concentration and recrystallization afforded cimetidine (14).

Cimetidine has a molecular weight of 252.34 and empirical formula of $C_{10}H_{16}N_6S$. It occurs as a white or whitish microcrystalline powder, It is soluble in 88 parts of water, the solubility is increased by dilute hydrochloric acid. Cimetidine has a melting range between 141° and 143° C(1,15).

Analytical methods

1.Non-aqueous Titration.

Non-aqueous titration is normally used as analytical method for basic compounds, so cimetidine can be analysed by dissolving in glacial acetic acid and then the solution is titrated with 0.1N perchloric acid, crystal violet TS is used as indicator, or can be determined the end point potentiometrically(5,6,7).

Titrimetric method offers an accurate, rapid, simple and inexpensive mean of assay but samples have to be in dry, powdered form, otherwise end point is not sharp.

2.Spectrophotometric method.

2.1 Ultraviolet absorption method.

According to the test methods for pharmaceutical development and bioavailability of cimetidine capsule and tablet formulations of Smith Kline and French Research Laboratories, cimetidine was determined by dissolving in 0.1N sulfuric acid and the solution was diluted to a concentration of about 10 microgram per millilitre. The absorption is read at 218 and 260 nm, the different between the two readings

being used to calculate the potency, E 1 %, 1 cm of cimetidine for the particular ultraviolet spectrophotometer is used (8).

The spectrophotometer used should have the ability to measure the absorbance accurately at wavelength lower than 220 nm(9).

2.2 Colorimetric method.

Mehta and Chainani(10) described the colorimetric procedure for the analysis of cimetidine via reaction with diazotized sulfanilic acid in alkaline medium. Cimetidine solution in 0.1N hydrochloric acid was diluted with distilled water and cooled in ice-bath, then treated with cooled diazotized sulfanilic acid and kept in the ice-bath for 30 minutes. Distilled water and sodium bicarbonate solution was added,kept in ice-bath and then at room temperature. A yellow-colored product of basic azo dyestuff with a maximum absorption at wavelength 405 nm was produced. This method is based on Ehrlich diazo reaction, the coupling of imidazole derivatives with diazotized sulfanilic acid occurs in the α-position to the imide group of the imidazole ring(11,12,16).
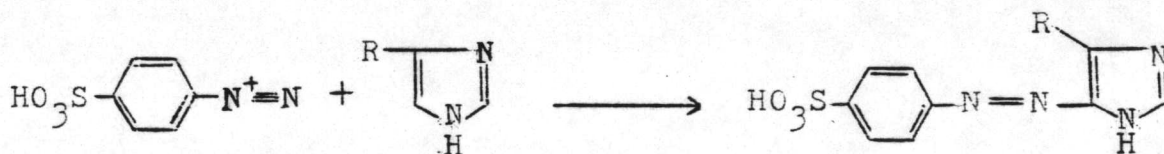
This method was relative time consuming. All reagents, standard and sample solution had to be cooled in ice-bath and the reaction had to be kept in ice-bath almost throughout the experiment. Moreover, it is disagree with the theory of Ehrlich diazo reaction. From the structural viewpoint, α-position to the imide group of imidazole ring of cimetidine was substituted by methyl group, so the diazotized sulfanilic acid could not react at this position.

3. Chromatographic method.

Liquid chromatography is a separation technique based on a sample partitioning between two phases; stationary phase of large surface area and mobile liquid which percolates over stationary bed. Prior to 1967, liquid column chromatography (LCC) separations were not normally carried out in an optimum manner: for this reason, the technique was viewed as slow and inefficient. After 1967, LCC has undergone dramatic changes and rivaled gas chromatography in speed of analysis and column efficiency. The modernized technique is commonly known as high pressure liquid chromatography(HPLC) (17-19).

The development of HPLC in 1969 proved to be very valuable in the analysis of drugs from pharmaceutical formulations and biological samples(20-22). HPLC has many advantages over conventional column chromatography. It is fast and versatile. It has high sensitivity, gives good resolution, and affords both quantitative and qualitative analysis. Furthermore, the sample is not destroyed and fraction can be collected. Also,

the sample size requirements are small and usually a complete analysis can be carried out on a few microliters of sample.

HPLC has many advantages over gas chromatography. In gas chromatography, the solute must volatile and thermally stable, whereas in liquid chromatography, high molecular weight compounds and those that are labile, polar or non-volatile can be separated. The main requirement is that the solute be soluble in mobile phase.

Basically, the apparatus of a HPLC system consists of a reservior for mobile liquid, pump, injection port, column, detector, recorder and thermostats for column and detector. More sophisticated models also include an integrator with printed readouts or an on-line computer in which case the areas are given in numerical values along with retention times.

To get the best results from liquid chromatographic system, it is important to choose the correct separation mode for the analysis desired, and the operating conditions for each group of compounds must be investigated and optimized. Separation mode. Separation modes used in liquid chromatographic analysis are:

1. Adsorption chromatography.

2. Reversed-phase chromatography.

3. Liquid-liquid chromatography.

4. Polar bonded-phase chromatography.

5. Ion-exchange chromatography.

6. Size exclusion (gel permeation) chromatography.

The choice for one of these has to be based upon the chemical criteria of the sample components as well as on the matrix of the sample.

1.Adsorption chromatography.

Adsorption chromatography has been used quite extensively in pharmaceutical HPLC analyses. The solid support used are silica and alumina which have polar hydroxyl group as active sites. They interact with polar functional group of sample and the components of sample is separated by selective interaction. Thin layer chromatography is considered to be an adsorption chromatography. The water content of the stationary phase is an important aspect in adsorption chromatography. In general,the activity of adsorbents is increasing with decreasing water content. Compound-retention is increasing with increasing adsorbent's activity and vice versa. Since the stationary phase is always in equilibrium with the mobile phase,the activity of the adsorbent is controlled by the water content of the mobile phase. It is therefore possible to optimize an adsorption-separation by changing the water content of the mobile phase. The mobile phase systems are most often binary mixtures of the organic solvents,such as chloroform,hexane,benzene,ethyl acetate isopropanal,methanol etc. This mode of chromatography has been used for compounds with low molecular weight($<$1000),low to moderate polarity,non ionic and good solubility in organic

solvents. The retention based upon the number and polarity of functional groups, dipole-moment and molecular size of elutes.

2. Reversed-phase chromatography.

Reversed-phase chromatography has also been used in many works relating to pharmaceutical analysis. The column packing materials have good chemical inertness, excellent stability, and recondition quickly in gradient elution procedures. They allow great freedom of choice with respect to solvents, solvent combinations and buffer solutions, providing a wide range of elution strength and mobile phase selectivity. The elution strength of the mobile phase increases with decreasing polarity of the solvent. This mode of chromatography has been used for compounds with moderate to high polarity, neutral and ionic soluble in water or polar solvents. Retention based upon hydrocarbon structure(alkyl-substitution, number and position of double bonds, ring structure), polar functional groups and molecular size.

3. Liquid-liquid chromatography.

Liquid-liquid chromatography is partition or solution chromatography. The sample is retained by partitioning between the mobile liquid and the stationary liquid. The requirement is that the mobile liquid not be a solvent for the stationary liquid. Infact, water is often used as the stationary liquid and organic solvents are used as the mobile liquid.

4.<u>Polar bonded-phase chromatography</u>.

Significant change in chromatographic selectivity of these phases can be observed with the variation of the polar functional groups permanently bonded onto the surface of porous silica- Amino, Diol, Cyano, Phenyl, Ether, Carbowax. These phases are most often used in normal mode with mobile phase systems,similar to those used in adsorption chromatography. In this mode, retention on these phases is usually lower than retention of the same compound on silica gel columns. In some cases, e.g. the analysis of highly water-soluble compounds, reversed phase system had also been used. Most of the developed polar bonded phases are stable to hydrolysis over a wide pH-range(2.2 to 8) and are not significantly sensitive to the water content of the mobile phase. They need only short re-equilibrium time, when using gradient-elution.

5.<u>Ion-exchange chromatography</u>.

Ion-exchange chromatography uses zeolites and synthetic organic and inorganic resins to perform chromatographic separations by an exchange of ions between the sample and the resins. Compounds which have ions with different affinities for the resin can be separated. In the past, it had been widely used for analysis of polar ionic drugs, but its importance is decreasing nowadays. A reason for this can be seen in the variety of parameters influencing an ion-exchange separation (type of resin, type of buffer, ion-strength, pH, temperature) resulting in time consuming procedures for method development.

6.Size exclusion(gel permeation) chromatography.

In this process, a uniform highly porous, non ionic gel e.g. Sephadex is used to separate materials according to their molecular size. The small molecules can enter into the polymer network and will be retarded, whereas the large molecules cannot enter into the polymer network, and will be swept out of the column. The elution order then, would be the largest molecules first, medium size next, and then the smallest.

Operating parameter. The operating parameters are:

1.Column and packing material.

HPLC column is an important part of the chromatograph. Columns can be divided into two types: high performance analytical columns which are preferably 1-6 mm i.d., and preparative columns which are larger. Column lengths can be vary from 0.5 to 30 feet. The tubing used can be polyethylene and stainless steel in a straight or coiled form. Silica gel and alumina are the most commonly used adsorbents. One approach uses small porous particles of less than 15 microns diameter. Resolution and capacity are improved by using the smallest particles possible.

Column efficiency. The plate theory of chromatography, envigases a chromatographic column as being composed of a series of discrete but continuous, narrow, horizontal layers called theoretical plates. At each plate, equilibration of the solute between the mobile and the stationary phase is assumed to take place. Movement of the solute and solvent is

then viewed as a series of stepwise transfers from one plate to the next.

The efficiency of a chromatographic column as a separation device increases as the number of equilibrations increases— that is, as the number of theoretical plates increases Thus, the number of theoretical plates N is used as a measure of column efficiency. A second term, the height equivalent of a theoretical plate H, also serves this purpose. The relationship between these two parameters is

$$N = \frac{L}{H}$$

where L is the length of the column packing. Note that H decreases as the efficiency of a column becomes greater. That is, as H becomes smaller, the number of equilibrations that occure in a given length of column becomes larger.

In the rate theory, the column efficiency is expressed in terms of the height equivalent of a theoretical plate H, which is defined as

$$H = \frac{\sigma^2}{L}$$

where L is the distance which a zone maximum has migrated down a column and σ is the standard deviation in units of column height. Thus, H represents the broadening expressed in terms of σ^2 (the variance) per unit length of column. Note that H becomes smaller as the column becomes more efficient (that is with zone broadening).

The efficiency of a column can also be expressed in terms of its number of theoretical plates N as

$$N = \frac{\sigma^2}{H^2}$$

Clearly, a large number of plates leads to high efficiency.

Column resolution. The ability of a column to resolve two solutes is of prime interest in chromatography. A quantitative term for expressing the resolving power of a column for solutes X and Y is the resolution R_s, which is defined as

$$R_s = \frac{\Delta Z}{W}$$

where Z is the separation (usually in units of time) when the second solute is exiting from the column. The zone front, peak, or tail, can be used as the reference point. The width of two adjacent peaks will ordinarily be approximately the same $(W_x = W_y = W)$. For the same kind of packing, the resolution can be improved by lengthening the column and thus increasing the number of theoretical plates.

2. Mobile phase.

In HPLC, whether organic or aqueous mobile phases are used, which was determined by the type of separation achieved and the kind of column utilized. Aqueous solution are used in ion-exchange chromatography and either aqueous or organic solvents or both are used in reversed- phase, adsorption, or gel-permeation chromatography.

In using a liquid chromatograph with a UV detector,

it is important that neither the mobile phases nor any impuri-
ties in them absorb strongly in the UV at the wavelength used
in the detector. The best type of mobile phases used in separa-
ting a group of compounds, must be determined for each specific
case. For selection of a good mobile phase, a number of gener-
ral criteria must be satisfied. The mobile phase should be pure,
be a good solvent for the sample, have a low viscosity, not al-
ter the stationary phase, be compatible with the detector, and
must be compatible with system components.

3.Stationary phase.

In partition chromatography, the stationary phase must
be insoluble in the mobile phase and distributed as a thin
film on the support. It can be bonded chemically to the sur-
face of the solid support to provide a thin film and prevent
bleeding. Adsorbents in liquid solid chromatography must exhi-
bit a uniform surface to avoid tailing and irreversible adsorp-
tion.

4.Type of elution.

4.1 Isocratic elution mode.

The isocratic elution mode is desirable for a simple
mixture because it is possibly to achieve the separation and it
makes the unnecessity to regenerate the column.

4.2 Gradient elution mode.

Gradient elution or solvent programming is the change
of solvent composition during a separation in which the sol-
vent strength increases from the beginning to the end of the

separation. It is well suited to the analysis of samples of unknown complexity since good resolution is autometically provided for a wide range of sample polarities.

5.Flow rate.

The flow rate of the column can be varied to obtain the best elution in the shortest possible time. In general, resolution can be improved by decreasing the flow rate, while higher speed is obtained by increasing the flow rate, and more injections can be made per unit time.

6.Pressure.

In HPLC, pressure in the range of 500-5,000 psi are required. Many parameters e.g. fluid viscosity, column length flow rate, particle diameter influence the pressure.

7.Temperature.

HPLC is done most conveniently at ambient temperature, but other temperatures can improve both speed and resolution.

8.Sample size and injection.

In most HPLC used for analytical purposes, the volume of solution and concentration of sample are very low. The volume used in most systems ranges from 1 to 30 μl, depending on the design and sensitivity of the instrument.

9.Detection.

It is important to choose the correct detector for the samples to be analysed, because the detector indicates elution of the separated components from the column and provides a measure of the amount of each component. Detectors commonly

used in HPLC are UV and visible absorption and refractive in-
dex. Fluorescence, flame ionization, polarography, radioacti-
vity, and heat of adsorption are also used (21,22).

The fixed wavelength UV-absorption detector(254 nm)
is the most widely used in pharmaceutical HPLC applications.
The sensitivity of the detector is sufficient for a majority
of compounds. For detection of compounds having only little
absorption at 254 nm or when high sensitivity of detection is
needed, a variable wavelength detector is the better choice.
In this case, a wavelength is chosen which is more specific
for the compounds to be analyzed. The selection of this wave-
length has to be done on the basis of three criteria:

1.The absorption spectrum of the compound to be ana-
lyzed.

2.The absorption of the mobile phase used.

3.Interference of other sample constituents.

Quantitative Analysis.

Quantitative analysis involves the whole process of
analysis from preparation of sample to interpretation of the
results. From an operation point of view, it is helpful to di-
vide the process into four steps:

1.Generation of detector signal.

This include sampling and sample preparation, sample
introduction, chromatographic process, detection and amplifi-
cation.

2.Conversion of the detector signal into numerical data.

The usual technique is to display the detector signal on a strip chart recorder and perform the conversion by measurement of peak hight, peak area, digital integrators and computers.

3.Conversion of the numerical data to composition.

The principle methods used to relate digital data to the composition of the sample are internal standardization and external standardization or calibration techniques. The internal standardization is used in analyses during pharmacokinetic and metabolism studies and clinical assays when trace analysis of drugs or metabolites in biological matric is performed. The external standard method is widely used in quality control of raw materials, drugs and formulations, where mostly main components are analyzed. Its main advantage is that only peaks of interest need to be calibrated and calculated.

4.Interpretation of the data.

The usual procedure is to express analytical results by means of the average and some measure of precision. The standard deviation has largely replaced other measures of precision because it is more statistically meaningful. (21-25)

To date, a relatively numerous number of practical applications of HPLC has been reported. Major areas of HPLC application in pharmaceutical analysis are product development and production control. In product development, HPLC is used

for control of synthesis, isolation of natural compounds, pharmacokinetics, metabolism, drug interaction, and stability testing. In production control, HPLC has an important role in quality control of raw material, drug, excipient and formulation (26-31).

Randolph et al (13) determined cimetidine in blood and urine by HPLC. Leonard et al (8) studied the bioavailability of cimetidine capsule and tablet formulations by HPLC. In both methods, cimetidine is extracted from biological fluids with 1-octanol and back-extracted into dilute acid and then into a small volume of ethanol by saturation with potassium carbonate. HPLC analysis was performed on a column of $5-\mu m$ silica with a mixed mobile phase consisting primarily of acetonitrile. UV-absorption was used as detector. The method measured concentrations of cimetidine as low as $0.05 \mu g/ml$.

The purpose of this thesis is to develop a convenient, faster and precise method which could be employed in quality control of cimetidine both in pharmaceutical dosage forms and raw material. In this thesis, the method used in quantitative determination of cimetidine was done by HPLC, using adsorption chromatography as separation mode and UV absorption as detector. The outline of this thesis was based on the following statements.

1. Solvent system used in HPLC was selected by testing solubility of cimetidine in different kinds of solvent and their mixture, followed by running thin layer chromatography.

The system which has proven to give the high Rf value was se-
lected. The other optimum conditions, such as wavelength of
UV absorption, flow rate, pressure and retention time are .
studied and selected for the assay of cimetidine in pharmaceu-
tical preparations.

2.In order to measure the accuracy and precision of
this method, standard cimetidine was added in a known amount
of cimetidine tablets and the percent recovery was determined
in comparison with the non- aqueous titration and spectrophoto-
metric method.

3.Apply this procedure to several commercial prepara-
tions containing cimetidine and its salt and the results ob-
tained are compared with those obtained by non-aqueous titra-
tion and spectrophotometric method.

The usefulness of the proposed method.

1.The new method is suitable for the analysis of ci-
metidine and its salt in the pharmaceutical preparations.
It is sensitive and time-saving, the results are reliable by
comparing well with non-aqueous titration and spectrophotome-
tric method.

2.The new method uses the technique of liquid chroma-
tography which is widely used and a helpful analytical tech-
nique since results is obtained easily and rapidly, moreover,
accurately and precisely.

3.The principle technique in quantitative analysis of

pharmaceutical compounds by HPLC is studied which has advantage in quality control works of another drugs.