CHAPTER II

MATERIALS AND METHODS

MATERIALS

All of the materials employed in this study were obtained from commercial sources.

ACTIVE RAW MATERIALS

1. Tetracycline Hydrochloride from
   - I, China National Metals & Minerals Co., Republic of China (Lot. No. 8904184)
   - II, Globe Chemical GMBH, Germany (Lot. No. 001-04326-89)
   - III, Sigma Chemical Company, U.S.A. (Lot. No. 68F0070)
2. Cimetidine from
   - I, Arcana Farmaceutica Co., Italy (Lot. No. 71214)
   - II, LEK Ljubljana Co., Yugoslavia (Lot. No. 45580189)
   - III, IL Dong Pharmaceutical Co., Ltd., Korea (Batch No. 0112)
   - IV, Cometlex Co., Ltd., Hungary (Batch No. 86295.01)
   - V, Juritex Import - Export GMBH, Germany (Batch No. 4000)
3. Mefenamic Acid from
   - I, Chin Toong Pharmaceutical Co., Korea (Batch No.91023)
   - II, Shaanxi China National Medicine & Health Product

Corporation, Republic of China (Lot. No. 860932)

- III, Sigma Chemical Company, U.S.A. (Lot. No. 29F0571)

OTHER MATERIALS

4. Lactose monohydrate (Wyndle, New Zealand)
5. Magnesium stearate (Pharmaceutical Science, Thailand)
6. Acetone (E. Merck, Germany)
7. Ether (E. Merck, Germany)
8. Absolute ethanol (E. Merck, Germany)
9. Potassium biphthalate (E. Merck, Germany)
10. Sodium hydroxide (E. Merck, Germany)
11. Glacial acetic acid (E. Merck, Germany)
12. Potassium phosphate, monobasic (E. Merck, Germany)
13. Hydrochloric acid (E. Merck, Germany)
14. Tetracycline Reference Standard 97.0% (The Armed Forces Pharmaceutical Factory, Thailand)

APPARATUS

1. Analytical balance (Sartorius, A 200S, Germany)
2. Spectrophotometer (The Bausch & Lomb, New York, U.S.A.)
3. Scanning electron microscope (JEOL, JSM-35CF, Japan)
4. Moisture determination balance (OHAUS, Scale Corp., U.S.A.)
5. Disintegration apparatus (Hanson Research model QC-21, U.S.A.)
6. Dissolution apparatus (Hanson Research, U.S.A.)
7. Cube Mixer (Erweka AR400, Germany)
8. Hot air oven (Mammertt, Germany)
9. Sieve shaker (Josef Deckelman, Germany)

10. Nest of sieve (Endecotts Ltd., London, England)
11. Magnetic stirrer (Sybron corporation, U.S.A.)
12. pH meter (Hanna instruments, U.S.A.)
13. Capsule filling machine (KSL, Bangkok Thailand)
14. Melting point apparatus (Buchi 520, Switzerland)
15. Infrared spectrophotometer (Fourier Transforom 1760X, Japan)

METHOD

1.Physical Properties Determinations of Active Raw Materials

1.1 Size and Shape from Scanning Electron Microscope

Samples of each active raw materials were coated with gold prior to the microscopic examination using ion sputtering and then photographed at appropriate magnification by scanning electron microscope.

1.2 Particle Size Distribution

Particle size distribution was determined by sieve analysis. The 100 g of each active raw materials was put on the top sieve of a sieve series ranging from 850, 425, 250, 180 to 150 µm respectively. The nest of sieve was placed on the sieve shaker for 10 minutes. The results, which averaged from two determinations were reported as percentage of weight retained on each sieve size.

1.3 Bulk Density, Tapped Density and Compressibility Determination

The bulk density and tapped density were determined from the weight of about 30 g sample (record accurate weight), carefully transferred into a 100 ml graduated cylinder and the bulk volume was recorded. Division of weight by bulk volume showed bulk density. Tapped density was performed by dropping graduated cylinder on a hard wood surface from a height of 5 cm until a constant volume was obtained. Division of weight by tapped volume showed tapped density. Both densities were averaged from 3 determinations. The compressibility index was calculated from the following equation (Hiestand and Peot, 1974)

$$\% \ Compressibility = \frac{(T-B)}{T} \times 100$$

where T and B are tapped and bulk density, respectively.

1.4 True Density Determination

True Density was determined by using a 50 ml pycnometer (Wertheim, Germany). The 5 g of the sample was accurately weighed and transferred into pycnometric bottle. Enough suitable solvent for each active raw materials was added to fill up the pycnometer, whipped excess droplets of the solvent, finally weighed the whole bottle and calculated true density as follow

$$True \ Density = \frac{W_1 . W_2}{50 (W_1 - W_3 + W_2)}$$

Where W_1 = solvent weight in pycnometer,

W_2 = sample weight in pycnometer,

W_3 = solvent & sample mixture weight in pycnometer

The result shown was averaged from 3 determinations.

1.5 Moisture Determination

The moisture content of active raw materials was determined by using OHAUS moisture determination balance. The 10 g of sample was exposed to an IR lamp set at 1.5 inch from the pan and intensity of approximately 150°C until constant weight was obtained. The percent moisture content was calculated from the following equation :

$$\% \textit{ Moisture Content} = \frac{\textit{Wet Weight} - \textit{Dry Weight}}{\textit{Wet Weight}} \times 100$$

1.6 Angle of Repose Determination

Angle of repose was determined by cylinder method. Appropriate amount of active raw materials was carefully filled in the cylinder, which was placed on the graph paper, until it was filled at the top of the cylinder, then slowly lifted the cylinder in the vertical way, producing round heap. Angle of repose was calculated from the following equation (Harwood and Pilpe, 1968)

Angle of repose = $\tan^{-1} H/R$

Where H and R are the height and radius of the heap, respectively.

1.7 Flowability Determination

Flow rate of the sample were obtained by the use of flowmeter. The flowmeter consists of plastic tube which has 38.1 mm in diameter and 45 cm in height, stand on plastic plate which has a circular orifice of 11 mm in diameter. The powder of active raw materials were full-filled into the plastic tube and then the plastic column was pulled over the orifice of the plastic plate. The powder of active raw materials were collected every 4 seconds intervals for 16 seconds. Amount of the powder of active raw materials flew through the orifice was plotted against times and flow rates were calculated from the slope.

1.8 Melting Range Determination

The Melting Range Determination of active raw materials were determined by using Melting Point Apparatus (Buchi, Model 520, Switzerland) and reported values were the average of three determinations.

1.9 Solubility Determination

Excess amounts of each active raw materials were mixed with measured amounts of water and absolute ethanol, respectively. Established equilibrium for each system at constant temperature and pressure to prepare complete saturated solutions then separated the undissolved active raw materials from the solutions by filtration and determined the concentration of active raw materials dissolved in the solutions by spectrophotometry. The reported value were the

average of two determinations.

1.9.1 Solubility of Tetracycline Hydrochloride in Water

Shook about 4 g of Tetracycline Hydrochloride samples with 25 ml of purified water for 30 minutes and filtered. Transfer 1 ml of the filtrate into 200 ml volumetric flask. Adjust with purified water and mixed. Then transfer 1 ml of the solution intc a 100 ml volumetric flask and diluted to the volume with purified water. The absorbance of the solution were determined spectrophotometrically in 1 cm cell at 276 nm. The amount of Tetracycline Hydrochloride dissolved was calculated from the calibration absorbance-concentration curve.

1.9.2 Solubility of Tetracycline Hydrochloride in Ethanol

Shook about 1 g of Tetracycline Hydrochloride samples with 25 ml of absolute ethanol for 30 minutes and filtered. Transfer 1 ml of the filtrate into 200 ml volumetric flask. Adjust with purified water and mixed. Then transfer 1 ml of the solution into a 10 ml volumetric flask and diluted to the volume with purified water. The absorbance of the solution were determined spectrophotometrically in 1 cm cell at 276 nm. The amount of Tetracycline Hydrochloride dissolved was calculated from the calibration absorbance-concentration curve.

1.9.3 Solubility of Cimetidine in Water

Shook about 1.5 g of Cimetidine samples with 100 ml of purified water for 24 hours and filtered. Transfer 1 ml of the filtrate into 100 ml volumetric flask. Adjust with purified water and mixed. Then transfer 1 ml of the solution into a 10 ml volumetric flask and diluted to the volume with purified water. The absorbance of the solution were determined spectrophotometrically in 1 cm cell at 218 nm. The amount of Cimetidine dissolved was calculated from the calibration absorbance-concentration curve.

1.9.4 Solubility of Cimetidine in Ethanol

Shook about 1.5 g of Cimetidine samples with 20 ml of absolute ethanol for 24 hours and filtered. Transfer 1 ml of the filtrate into 100 ml volumetric flask. Adjust with ethanol and mixed. Then transfer 1 ml of the solution into a 100 ml volumetric flask and diluted to the volume with purified water. The absorbance of the solution were determined spectrophotometrically in 1 cm cell at 218 nm. The amount of Cimetidine dissolved was calculated from the calibration absorbance-concentration curve.

1.9.5 Solubility of Mefenamic Acid in Ethanol

Shook about 0.5 g of Mefenamic Acid samples with 100 ml of absolute ethanol for 24 hours and filtered. Transfer 1 ml of the filtrate into 100 ml volumetric flask. Adjust with ethanol and mixed. Then transfer 2 ml of the solution into a 10 ml volumetric flask and diluted to the volume with ethanol. The absorbance of the solution were

determined spectrophotometrically in 1 cm cell at 280.5 nm. The amount of Mefenamic Acid dissolved was calculated from the calibration absorbance-concentration curve.

1.10 The IR Spectroscopy

The IR spectra of all active raw materials were recorded on a KBr disc with an infrared spectrophotometer (FT-IR Model 1760, Japan).

2. Determination of % Content of Active Raw Materials

2.1 Tetracycline Hydrochloride

Preparing the standard solution at the concentration 2, 4, 6, 8, 10, 12 µm/ml in purified water by using Tetracycline Hydrochloride Reference Standard.The absorbance was measured at 276 nm. by spectrophotometer. Then plot the standard concentration absorbance curve of Tetracycline Reference Standard. Calculate % content of Tetracycline Hydrochloride I, II and III at 12 µm/ml from the regression line.

2.2 Cimetidine

Dissolved 300 mg in glacial acetic acid 20 ml and 2 drops of 1% crystal violet solution (in glacial acetic acid) and titrated with 0.1 N perchloric acid VS in glacial acetic. A blank was run to confirm non-interference, if any. Each ml of 0.1 N perchloric acid VS is equivalent to 25.23 mg of Cimetidine and the reported value were the average of two determinations.

2.3 Mefenamic Acid

Dissolved 0.6 g in 100 ml of warm absolute ethanol previously neutralized to phenol red solution and titrate with 0.1 M sodium hydroxide VS using phenol red solution as indicator. Each ml of 0.1M sodium hydroxide VS is equivalent to 0.02413 g of Mefenamic Acid and the reported value were the average of two determinations.

3. Preparation of Tetracycline Hydrochloride, Cimetidine and Mefenamic Acid Capsules and Their Fixed-Diluent Formulas Capsules

The following formulas in Table 1 were used in preparing Tetracycline Hydrochloride, Cimetidine and Mefenamic Acid capsules. The quantities were for one capsule. The formulas in Table 2 were used in preparing Fixed-diluent Formula of Tetracycline Hydrochloride, Cimetidine and Mefenamic Acid Capsules.

Each ingredients was passed individually through a 40-mesh sieve to break up any agglomerate. Then the mixture were blended by geometric dilution technique. The powder mixture was transferred to a cube mixer and mixed for 15 minutes to ensure homogeneity. The powder mixture were filled into capsules no.1 by using a capsule filling machine.

To study the effect of variation of active raw materials on physical properties of hard gelatin capsule dosage form, the Fixed-diluent Formulas were performed.

Table 1

The Formulations of Experimental Capsules

Active Raw Materials (mg/capsule)		Lactose	Magnesium Stearate
Tetracycline I	250	qs.	2%
Tetracycline II	250	qs.	2%
TetracyclineIII	250	qs.	2%
Cimetidine I	200	qs.	2%
Cimetidine II	200	qs.	2%
Cimetidine III	200	qs.	2%
Cimetidine IV	200	qs.	2%
Cimetidine V	200	qs.	2%
Mefenamic I	200	qs.	2%
Mefenamic II	200	qs.	2%
Mefenamic III	200	qs.	2%

Table 2

The Fixed-Diluent Formulas of Experimental Capsules

Active Raw Materials (mg/capsule)		Lactose	Magnesium Stearate
Tetracycline I	250.00	A**	2%
TetracyclineII*	250.00	A**	2%
TetracyclineIII	250.00	A**	2%
Cimetidine I*	200.00	B**	2%
Cimetidine II	200.00	B**	2%
Cimetidine III	200.00	B**	2%
Cimetidine IV	200.00	B**	2%
Cimetidine V	200.00	B**	2%
Mefenamic I	200.00	C**	2%
Mefenamic II	200.00	C**	2%
Mefenamic III*	200.00	C**	2%

* used as working standard

** actual weight of working standard

4. Evaluation the Physical Properties of Tetracycline Hydrochloride, Cimetidine and Mefenamic Acid Capsules

4.1 Weight Variation of Tetracycline Hydrochloride Capsules, Cimetidine Capsules and Mefenamic Acid Capsules and their Fixed-Diluent Formulas.

Twenty capsules of each active raw materials capsules were weighed individually using an analytical balance. Care was taken to preserve the identity of each capsule and remove the contents of each capsule with the aid of a small brush or pledget of cotton. The emptied shell were weighed individually. The net weight of its contents were calculated by subtracting the weight of the shell from the respective gross weight. The average weight and standard deviation of each active raw materials capsules were calculated.

4.2 Content Uniformity

4.2.1 Tetracycline Hydrochloride Capsules and their Fixed-Diluent Formulas.

Sample of ten capsules were assayed individually. One capsule was placed in a 100-ml volumetric flask then added about 50 ml of purified water and allowed to disintegrate for 20 to 30 minutes with occasionally swirling. The volumetric flask was adjusted with purified water to 100 ml, mixed and filtered. Dilute a suitable aliquot of the subsequent filtrate quantitatively and stepwise with purified water so that the concentration of tetracycline hydrochloride

is about 12.5 µg per ml. Measuring the absorbance of resulting solution at 276 nm and calculated the content of tetracycline hydrochloride from the standard curve shown in Figure 2.

4.2.2 Cimetidine Capsules and their Fixed-Diluent Formulas.

Sample of ten capsules were assayed individually. One capsule was placed in a 100-ml volumetric flask then added about 50 ml of purified water and allowed to disintegrate for 20 to 30 minutes with occasionally swirling. The volumetric flask was adjusted with purified water to 100 ml, mixed and filtered. Dilute a suitable aliquot of the subsequent filtrate quantitatively and stepwise with purified water so that the concentration of cimetidine is about 5 µg per ml. Measuring the absorbance of resulting solution at 218 nm and calculated the content of cimetidine from the standard curve shown in Figure 3.

4.2.3 Mefenamic Acid Capsules and their Fixed-Diluent Formulas.

Sample of ten capsules were assayed individually. One capsule was placed in a 1000-ml volumetric flask then added about 500 ml of phosphate buffer pH 7.8 and allowed to disintegrate for 20 to 30 minutes with occasionally swirling. The volumetric flask was adjusted with purified water to 1000 ml, mixed and filtered. Dilute a suitable aliquot of the subsequent filtrate quantitatively and stepwise with phosphate buffer pH 7.8 so that the concentration of

mefenamic acid is about 10 µg per ml. Measuring the absorbance of resulting solution at 283.5 nm and calculated the content of mefenamic acid from the standard curve shown in Figure 4.

4.3 Disintegration Time of Tetracycline Hydrochloride Capsules, Cimetidine Capsules and Mefenamic Acid Capsules and their Fixed-Diluent Formulas.

The disintegration apparatus according to USP XXII method was used to determine the disintegration time of each active raw materials capsules. One capsule was placed in each of the six tubes of the basket. The apparatus was operated, using purified water maintained at 37±2° C as the immersion fluid. The capsules were observed within the time limit of 20 minutes and the average was calculated from six determination.

4.4 Dissolution Studies

Dissolution profiles and dissolution rates was determined by using apparatus according to USP XXII. The average was calculated from three determinations.

4.4.1 Raw material of Tetracycline Hydrochloride , Tetracycline Hydrochloride Capsule and their Fixed-Diluent Formulas.

The dissolution rate of single capsule was measured in 900 ml of purified water at 37±0.5 °C as the dissolution medium. A capsule was placed on the vessel and the paddle was rotated at 75 rpm. Sample of 5 ml were withdraw periodically at 1, 3, 5, 7, 10, 15, 20, 30, 45, 60, and 90 minutes interval. The volume taken was substituted by an equal

volume of prewarm purified water. After suitable diluted with purified water, the sample was then assayed spectrophotometrically by measuring the absorbance at 276 nm and the concentration was calculated from standard curve presented in Figure 2.

4.4.2 Raw material of Cimetidine, Cimetidine Capsule and their Fixed-Diluent Formulas.

The dissolution rate of single capsule was measured in 900 ml of purified water at 37±0.5 °C as the dissolution medium. A capsule was placed on the vessel and the basket was rotated at 100 rpm. Sample of 5 ml were withdraw periodically at 1, 3, 5, 7, 10, 15, 30, and 45 minutes interval. The volume taken was substituted by an equal volume of prewarm purified water. After suitable diluted with purified water, the sample was then assayed spectrophotometrically by measuring the absorbance at 218 nm and the concentration was calculated from standard curve presented in Figure 3.

4.4.3 Raw material of Mefenamic Acid, Mefenamic Acid Capsule and their Fixed-Diluent Formulas.

The dissolution rate of single capsule was measured in 900 ml of phosphate buffer pH 7.8 at 37±0.5 °C as the dissolution medium. A capsule was placed on the vessel and the paddle was rotated at 100 rpm. Sample of 5 ml were withdraw periodically at 1, 3, 5, 7, 10, 15, 20, 25, 30, 40, 50, 60, 75, 90, 105, and 120 minutes interval. The volume

taken was substituted by an equal volume of prewarm phosphate buffer. After suitable diluted with phosphate buffer, the sample was then assayed spectrophotometrically by measuring the absorbance at 283.5 nm and the concentration was calculated from standard curve presented in Figure 4.

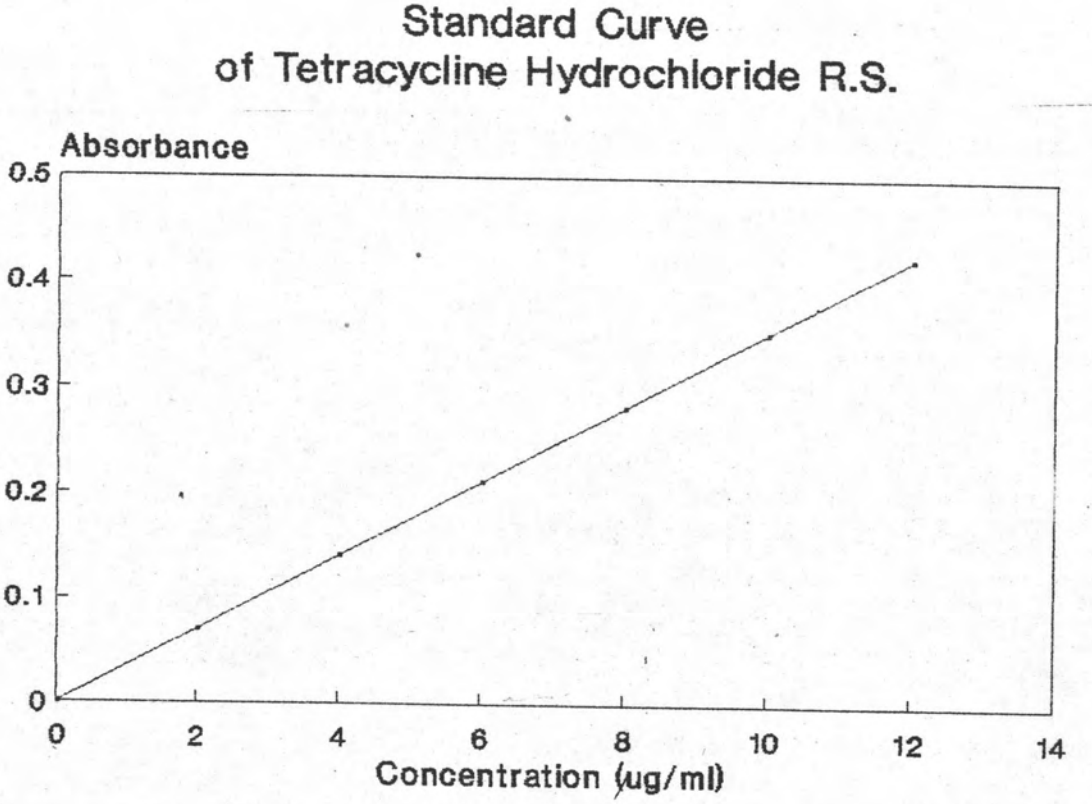

Figure 2 Standard Curve of Tetracycline Hydrochloride R.S. in water at 276 nm ($Y = 0.0362X + 0.0063$, $r^2 = 0.9993$)

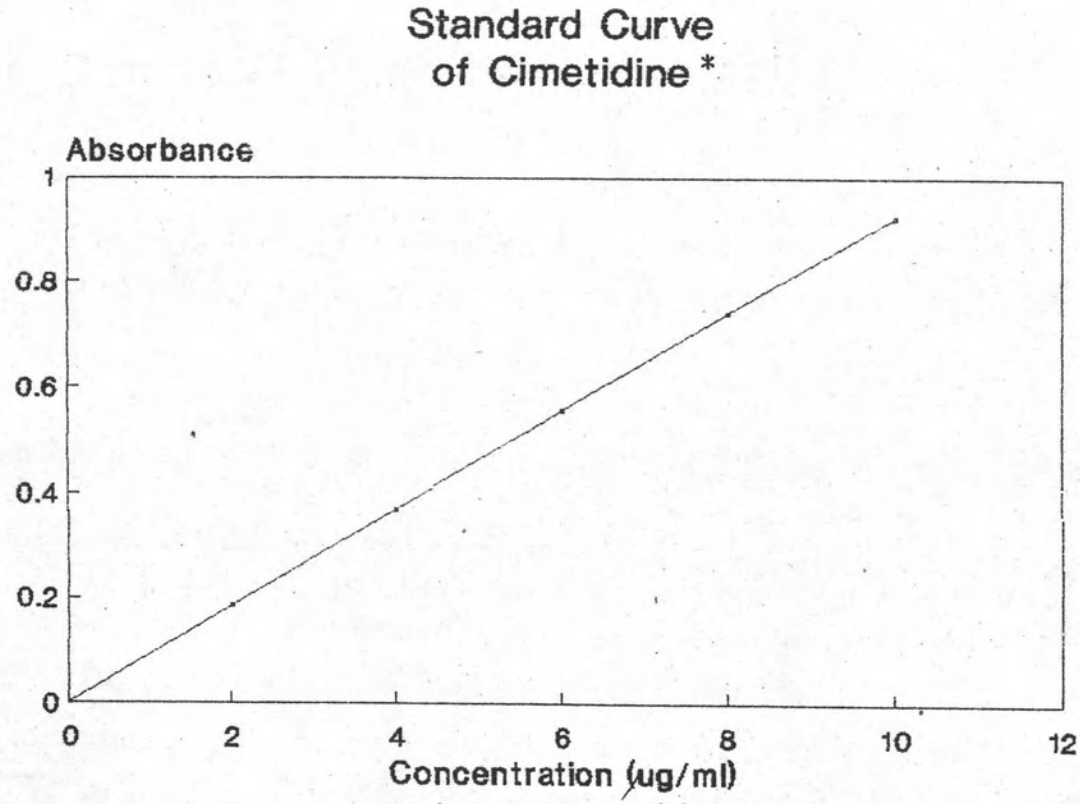

Figure 3 Standard Curve of Cimetidine in water at 218 nm ($Y = 0.0923X + 0.0106$, $r^2 = 0.9986$)

* The spectrophotometric method could be used for determination of cimetidine in pharmaceutical preparations compared well with non-aqueos titration and high pressure liquid chromatographic method (Nidapan Ruangrittinon, 1982).

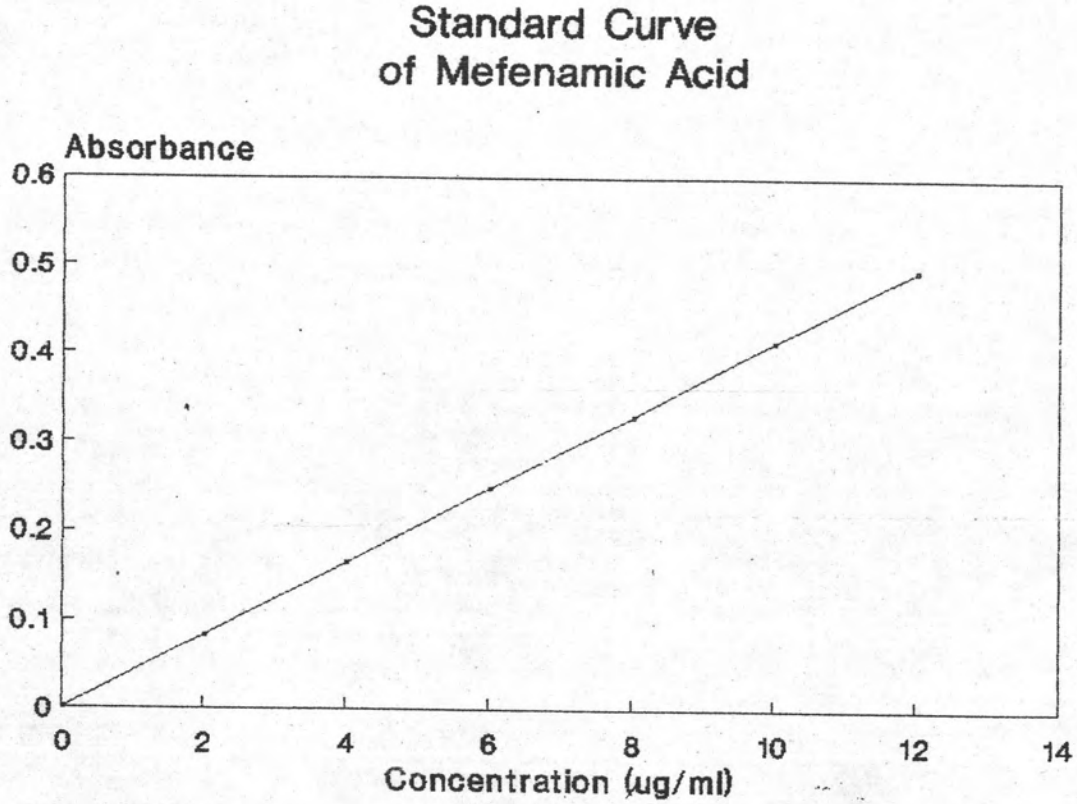

Figure 4 Standard Curve of Mefenamic Acid in Phosphate Buffer pH 7.8 at 283.5 nm ($Y = 0.0413X - 0.0032$, $r^2 = 0.9999$)