CHAPTER II

Experimental

1. Materials

The following meterials obtained from commercial sources were used as received

1.1 Model Drug

Diclofenac Sodium (Yung-Zip Chemical Ind., Taiwan)

Diclofenac Diethylamine (Yung-Zip Chemical Ind, Taiwan; supported by Siam Pharmaceutical Co Ltd. Thailand)

1.2 Base

Castor oil (Japan, supplied by Pharmaceutical science LTD, Part., Thailand)

Isopropyl myristate (German, supplied by S.Tong Chemical Co., Ltd, Thailand)

Mineral oil (USA, supplied by Srichand-United Dispensary Ltd., Thailand)

Silicone oil No. 350 (USA, suppplied by S. Tong Chemical Co., Ltd., Thailand)

Carbomer 940 (Carbopol 940, USA, supplied by S.Tong Chemical Co.,Ltd, Thailand)

Polyethylene glycol 1000 monocetyl ether (Cetomocrogol 1000,England, supplied by S.Tong Chemical Co., Ltd., Thailand)

Cetyl alcohol (Henkel, German, supplied by Srichand-United Dispensary Ltd., Thailand)

Glycerin (supplied by Srichand-United Dispensary Ltd., Thailand)

Isopropyl alcohol (E. Merck, Germany)

PEG-40-hydrogenated castor oil (German,supplied by S. Tong Chemical Co., Ltd., Thailand)

Poloxamer 407 (Pluronic F127, supported by BASF (Thailand))

Propylene glycol (Arco, USA, supplied by Srichand-United Dispensary Ltd., Thailand)

Sodium carboxymethyl cellulose, high viscosity 1800 (supplied by Pharmaceutical Traders Co., Ltd., Thailand)

Sodium hydroxide (Eka Nobel, Sweden)

Sorbitol solution (supplied by Srichand-United Dispensary Ltd., Thailand)

Sorbitan monolaurate (Span 20,Japan, supplied by Srichan-United Dispensary Ltd., Thailand)

Sorbitan monostearate (Span 60,Japan, supplied by Srichan-United Dispensary Ltd., Thailand)

Sorbitan monooleate (Span 80, Japan,supplied by Srichan-United Dispensary Ltd., Thailand)

Spermaceti (Japan, supplied by Srichan-United Dispensary Ltd., Thailand)

Stearic acid (German, supplied by Srichan-United Dispensary Ltd., Thailand)

Triethanolamine (E. Merck, Germany)

Polyoxyethylene 20 sorbitan monolaurate (Tween 20, Japan, supplied by Srichan-United Dispensary Ltd., Thailand)

Polyoxyethylene 80 sorbitan monooleate (Tween 80, Japan, supplied by Srichan-United Dispensary Ltd., Thailand)

White beeswax (Japan,supplied by Pharmaceutical science LTD, Part., Thailand)

White soft paraffin B.P. (supplied by G.P.O., Thailand)

1.3 Miscellaneous

Acetic acid, glacial (E. Merck, Germany)

Chloroform (E. Merck, Germany)

Dihydrogen potassium phosphate (E. Merck, Germany)

Di-sodium hydrogen phosphate (E. Merck, Germany)

Hydrochloric acid (E. Merck, Germany)

Mefenamic acid (Sigma, USA)

Methanol, HPLC grade (E. Merck, Germany)

Mono-sodium hydrogen phosphate (E. merck, Germany)

Potassium hydrogen phthalate (E. Merck, Germany)

Propyl paraben (Japan, supplied by Srichand-United Dispensary Ltd., Thailand)

Water, 3 times distilled

2. Instruments

Analytical balance, Sartorius model A200S (Germany)

High Performance liquid chromatography apparatus (USA)

- Milton Roy CM 4000 multiple solvent delivery system
- Milton Roy SM 4000 programmable wavelength detector
- Milton Roy CI-4100 intregrator

Dissolution apparatus, Hanson Research SR2 (USA)

Spectrophotometer, Bausch & Lomb Spectronic 2000 (USA)

pH meter, Hanna instruments 8417 (USA)

Hot plate and stirrer, Sybron/Thermolyne Nuova 7 (USA)

Scanning electron microscope, JSM-T220A, JEOL (Japan)

Infrared spectrophotometer, Shimadsu-IR 440 (Japan)

3. Preformulation Studies of Drug Powders

3.1 Determination of the Powder Characteristics

Photomicrographs of each drug powders were taken by scanning electron microscope (JSM-T220 A, JEOL JAPAN). The sample was coated with gold prior to microscope examination using ion sputtering. Size, shape and surface of the drug powders were evaluated.

3.2 The Infrared Spectroscopy

The IR spectra of the drug powders were recorded on a KBr (Potassium bromide) disc with an infrared spectrophotometer (Shimadzu-IR 440, Japan).

3.3 Determination for the Solubility of Drug

Drug was tested for the solubility in different solvent,(buffer pH 5, 6, 7, 8 and 9, propylene glycol, glycerin, sorbitol solution, isopropyl alcohol, ethanol and methanol).

Approximately 10 g. of drug was weighed into a 125 mL erlenmyer flask. Fifty mL of solvent was added. The erlenmyer flask was then closed with parafilm and aluminium foil, the mixture was magnetically stirred at a speed of 30 rpm at 35 ^{o}C for 12 hours. The flask was then stored in 35^{o}C incubator chamber and sampling every 3 hours until the content of drug was constant. The mixture was filtered through a Whatman paper no. 1, then discarding the first 10 mL. The filtrate was diluted to optimum concentration. The amount of drug was determined by High-Performance Liquid Chromatography (HPLC) method.

4. Stability Study of the Drugs

4.1 Preparation of Buffer Solution

Sorensen phosphate buffer was prepared with two separate stock solutions, monobasic sodium phosphate solution and dibasic sodium phosphate solution.

A 8.0 g. of anhydrous monobasic sodium phosphate was accurately weighed into 1000 mL volumetric flask which contained 200 mL of water. The flask was swirled until monobasic sodium phosphate was completely dissolved. The solution was adjusted to 1000 mL volume with water, and used as monobasic sodium phosphate stock solution. A 9.47 g. of anhydrous dibasic sodium phosphate was accurately weighed into another 1000 mL volumetric flask. Dibasic sodium phosphate was dissolved and adjusted to volume with water. The final solution was used as dibasic sodium phosphate stock solution.

A pH 5, 6, 7, 8 and 9 buffer solution was prepared by mixing monobasic sodium phosphate stock solution and dibasic sodium phosphate stock solution with optimum ratio to the desirable pH using pH meter. The buffer was then filtered throught a 0.45 micron pore size membrane.

4.2 Preparation of Drug Solution

An approximate 25 mg of drug was accurately weighed into five separate 500 mL volumetric flasks. The drug was individually dissolved and adjusted to volume with buffer solutions at pH 5, 6, 7, 8 and 9. The solution was filtered through membrane. Eight mL of filtrate was filled into 10 mL ampule. The sealed ampules were tested for leak test with vacuum methods.

4.3 Accelerated Stability Study of Drug

The sealed ampules of drug solution at every pH were divided into 4 groups, and stored in 35 ^{o}C, 45 ^{o}C, 55 ^{o}C and 65 ^{o}C incubators.

At the storage time intervals of 0, 0.5, 1, 2, 3, 4 and 5 months, the two sealed ampules were sampled from each incubator and analysed for drug content by HPLC method.

5. Formulations

5.1 Preparation of Creams

Each ingredient of the cream formulation was accurately weighed in the percentage ratio described in each formulation in Table 1 and 2. The drug was dissolved

Table 1 Composition of DS Creams

Formulation	1	2	3	4	5	6	7	8	9	10
Oil Phase										
Mineral oil	20	2	2	10	5	5	5	5	4	4
Stearic acid	20	20	20	5	5	-	-	-	5.24	10
White beeswax	-	-	-	5	1	-	-	-	2.62	5
WSP	-	-	-	5	-	-	-	-	-	-
Stearyl alc.	-	-	-	5	9	7	7	12.83	2	2
Cetyl alc.	-	-	-	-	-	5	5	9.17	-	-
Spermaceti	-	-	-	-	-	-	-	-	3.14	6
Ceto. 1000	-	-	-	-	-	3	3	3	3	3
Span 80	1	-	-	-	-	-	-	-	-	-
Span 60	-	-	-	2.03	0.5	-	-	-	-	-
Span 20	-	0.26	0.26	-	-	-	-	-	-	-
Water Phase										
PG	14	14	14	14	14	14	14	14	14	14
Sor. Sol.	-	5	5	-	-	-	-	-	-	-
Glycerin	-	6	-	-	20	10	-	-	-	-
Tween 80	1	-	-	0.97	2.5	-	-	-	-	-
Tween 20	-	3.74	3.74	-	-	-	-	-	-	-
DS	1	1	1	1	1	1	1	1	1	1
Buffer q.s.	100	100	100	100	100	100	100	100	100	100

Remark : Buffer = pH 7 buffer solution

Table 2 Composition of DE Cream

Formulation	11	12	13	14	15	16	17	18	19	20
Oil Phase										
Mineral oil	20	2	2	10	5	5	5	5	4	4
Stearic acid	20	20	20	5	5	-	-	-	5.24	10
White beeswax	-	-	-	5	1	-	-	-	2.62	5
WSP	-	-	-	5	-	-	-	-	-	-
Stearyl alc.	-	-	-	5	9	7	7	12.83	2	2
Cetyl alc.	-	-	-	-	-	5	5	9.17	-	-
Spermaceti	-	-	-	-	-	-	-	-	3.14	6
Ceto. 1000	-	-	-	-	-	3	3	3	3	3
Span 80	1	-	-	-	-	-	-	-	-	-
Span 60	-	-	-	2.03	0.5	-	-	-	-	-
Span 20	-	0.26	0.26	-	-	-	-	-	-	-
Water Phase										
PG	14	14	14	14	14	14	14	14	14	14
Sor. Sol.	-	5	5	-	-	-	-	-	-	-
Glycerin	-	6	-	-	20	10	-	-	-	-
Tween 80	1	-	-	0.97	2.5	-	-	-	-	-
Tween 20	-	3.74	3.74	-	-	-	-	-	-	-
DE	1.16	1.16	1.16	1.16	1.16	1.16	1.16	1.16	1.16	1.16
Buffer q.s.	100	100	100	100	100	100	100	100	100	100

Remark : Buffer = pH 7 buffer solution

in propylene glycol. All ingredients in the oil phase and ingredients in the aqueous phase except propylene glycol were separately heated to 75 ^{o}C and 78 ^{o}C on water bath, respectively. The water phase was then slowly added to the oil phase and stirred for 15 minutes then cooled to 50 ^{o}C after that the drug which was dissolved in propylene glycol was incorporated. The mixture was further mixed for 10 minutes and cooled to room temperature.

5.2 Preparations of Carbopol Gels

For an even number formulation in Table 3 and every formulation in Table 4, the drug was accurately weighed and dissolved in propylene glycol. Carbopol 940 was weighed and dispersed in buffer pH 7. The mixture of carbopol and water was stirred with magnetic stirrer for 6 hours in order to hydrate carbopol. The mixture was neutralized with either 10% NaOH or with triethanolamine. The carbopol gel was mixed for about 10 minutes and stored at room temperature for about 1 hour. Propylene glycol or drug in propylene glycol was added to carbopol gel and mixed for another 10 minutes. Isopropyl alcohol was then added to carbopol gel. Gel was adjusted to the accurate weight with buffer pH 7 and further mixed for about 5 minutes.

Table 3 Composition of DS Carbopol Gels

Ingredients	Formula										
	21	22	23	24	25	26	27	28	29	30	31
Carbopol	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.75	0.75	1
10% NaOH	2	2	2	2	2	2	2	2	3	3	4
TEA	-	-	-	-	-	-	-	-	-	-	-
PG	10	10	10	10	20	20	25	25	25	25	25
IPA	3.9	3.9	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.8
DS	-	1	-	1	-	1	-	1	-	1	-
Buffer to	100	100	100	100	100	100	100	100	100	100	100

Ingredients	Formula										
	32	33	34	35	36	37	38	39	40	41	42
Carbopol	1	0.5	0.5	0.5	0.5	0.5	0.5	0.75	0.75	1	1
10% NaOH	4	-	-	-	-	-	-	-	-	-	-
TEA	-	0.84	0.84	0.84	0.84	0.84	0.84	1.34	1.34	1.79	1.79
PG	25	10	10	20	20	25	25	25	25	25	25
IPA	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.8
DS	1	-	1	-	1	-	1	-	1	-	1
Buffer to	100	100	100	100	100	100	100	100	100	100	100

Remark : Buffer = pH 7 buffer solution

Table 4 Composition of DE Carbopol Gels

Ingredients	Formula									
	43	44	45	46	47	48	49	50	51	52
Carbopol	0.5	0.5	0.5	0.75	1	0.5	0.5	0.5	0.75	1
10% NaOH	2	2	2	3	4	-	-	-	-	-
TEA	-	-	-	-	-	0.84	0.84	0.84	1.34	1.79
PG	10	20	25	25	25	10	20	25	25	25
IPA	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.8
DE	1.16	1.16	1.16	1.16	1.16	1.16	1.16	1.16	1.16	1.16
Buffer to	100	100	100	100	100	100	100	100	100	100

Remark : Buffer = pH 7 buffer solution

Table 5 Composition of DS and DE Hydrophilic Gels

Ingredients	Formula										
	53	54	55	56	57	58	59	60	61	62	63
Sod. CMC	1.5	2	-	-	-	-	1.5	2	-	-	-
Poloxamer	-	-	20	20	25	30	-	-	20	25	30
PG	14	14	14	14	14	14	14	14	14	14	14
IPA	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.8
DS	1	1	-	1	1	1	-	-	-	-	-
DE	-	-	-	-	-	-	1.16	1.16	1.16	1.16	1.16
Buffer to	100	100	100	100	100	100	100	100	100	100	100

Remark : Buffer = pH 7 buffer solution

5.3 Preparations of Sodium Carboxymethylcellulose Gel

Each ingredient of sodium CMC gel formulation was shown in Table 5. Drug was accurately weighed and dissolved in propylene glycol. Sodium CMC was weighed and dispersed in buffer pH 7. The mixture of sodium CMC and water was magnetically stirred for about 10 hours. Then stored at room temperature for another 12 hours. Drug in propylene glycol was added to sodium CMC gel and mixed for 10 minutes. Isopropyl alcohol was then added to sodium CMC gel. Gel was adjusted to accurate weight with buffer pH 7 and mixed for 5 minutes.

5.4 Preparation of Poloxamer Gels

Each ingredient of poloxamer gel formulation was shown in Table 5 . Drug was accurately weighed and dissolved in propylene glycol except for Formulation 55. Poloxamer 407 was weighed and dispersed in buffer pH 7. The mixtures was stored at 0 ^{o}C and stirred every 10 minutes until poloxamer was completely dissolved. The poloxamer gel was stored at 0 ^{o}C for about 24 hours. The poloxamer gel was then kept at room temperature for about 3 hours. Propylene glycol or drug in propylene glycol was added into gel and mixed for about 10 minutes. Isopropyl alcohol was added to gel. Gel was adjusted to the accurate weight with buffer pH 7 and mixed for 5 minutes.

5.5 Preparation of Emulsions

Table 6 showed the ingredients of various emulsion formulations. Diclofenac was accurately weighed and dissolved in propylene glycol. Each emulsion formulations were prepared by 3 different methods.

Method 1

An oil phase and Span 20 were accurately weighed into a dry motar. Sodium CMC was accurately weighed and dispersed into the oil and Span 20 mixture.The mixture of Tween 20 and buffer pH 7 was then slowly added into motar and mixed. The drug dissolved in propylene glycol and isopropyl alcohol was then slowly added into a motar and mixed.

Method 2

An oil phase and Span 20 were accurately weighed into a dry motar. The mixture of Tween 20 and buffer pH 7 was slowly added into a motar and mixed. Sodium CMC powder was then slowly bestrewn into motar and mixed. The drug dissolved in propylene glycol and isopropyl alcohol was then slowly added and mixed.

Method 3

Sodium CMC was weighed and slowly dispersed into buffer pH 7 in the motar. Sodium CMC gel was stored at room temperature for about 3 hours. An oil and Span 20 were

Table 6 Composition of DS and DE Emulsions

Ingredients	Formula							
	64	65	66	67	68	69	70	71
Isopropyl myristate	20	-	-	-	20	-	-	-
Silicone oil	-	20	-	-	-	20	-	-
Mineral oil	-	-	20	-	-	-	20	-
Caster oil	-	-	-	20	-	-	-	20
Span 20	2.36	3.33	3.33	1.40	2.36	3.33	3.33	1.40
Tween 20	1.64	0.67	0.67	2.60	1.64	0.67	0.67	2.60
Sodium CMC	1	1	1	1	1	1	1	1
Propylene Glycol	14	14	14	14	14	14	14	14
Isopropyl Alcohol	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.8
DS	1	1	1	1	-	-	-	-
DE	-	-	-	-	1.16	1.16	1.16	1.16
pH 7 Buffer to	100	100	100	100	100	100	100	100

Table 7 Composition of DS Oil-Water Gels

Ingredients	Formula									
	72	73	74	75	76	77	78	79	80	81
Poloxamer 407	14	14	14	14	14	14	14	11	11	11
PHC	18	18	18	18	18	18	18	18	18	18
Silicone oil	-	5	10	-	-	-	-	-	-	-
Mineral oil	-	-	-	5	10	-	-	-	-	-
Caster oil	-	-	-	-	-	5	10	-	4	8
PG	14	14	14	14	14	14	14	14	14	14
IPA	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.8
DS	1	1	1	1	1	1	1	1	1	1
pH 7 Buffer to	100	100	100	100	100	100	100	100	100	100

weighed, mixed together and slowly added into a motar with continueously mixed. Drug in propylene glycol and isopropyl alcohol was then slowly added into motar and mixed.

The emulsion preparations from every method were homogenized and then filled in tightly closed aluminium tubes.

5.6 Preparations of Oil-Water Gels

Each ingredient of the oil-water gel formulations were shown in Table 7 and 8. Drug was weighed and dissolved in propylene glycol. PHC and oil except for Formulation 72 and 82 was weighed, mixed together and stored at 35 ^{o}C. The poloxamer gel containing drug was prepared as in 5.4. PHC and oil mixture, or PHC for Formulation 72 and 82, and isopropyl alcohol was slowly added to the gel and mixed. Oil-water gel was adjusted to accurate weight with buffer pH 7 and mixed for 5 minutes.

All preparations thus prepared were then filled into aluminium tubes and tightly closed until further evaluation.

Table 8 Composition of DE Oil-Water Gels

Ingredients	Formula									
	82	83	84	85	86	87	88	89	90	91
Poloxamer 407	14	14	14	14	14	14	14	11	11	11
PHC	18	18	18	18	18	18	18	18	18	18
Silicone oil	-	5	10	-	-	-	-	-	-	-
Mineral oil	-	-	-	5	10	-	-	-	-	-
Caster oil	-	-	-	-	-	5	10	-	4	8
PG	14	14	14	14	14	14	14	14	14	14
IPA	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.8
DE	1.16	1.16	1.16	1.16	1.16	1.16	1.16	1.16	1.16	1.16
pH 7 buffer to	100	100	100	100	100	100	100	100	100	100

6. In-vitro Evaluation of Prepared Preparation

6.1 Preparation Appearance

Color, clarity, texture, phase separation of each preparations were visually examined both initially and after freeze-thaw cycle. Only preparations of good appearance and no phase separation were subjected to freeze-thaw cycle study. These preparations were alternately stored, in a freezer for 24 hours and then 45°C for 24 hours for 5 cycles.

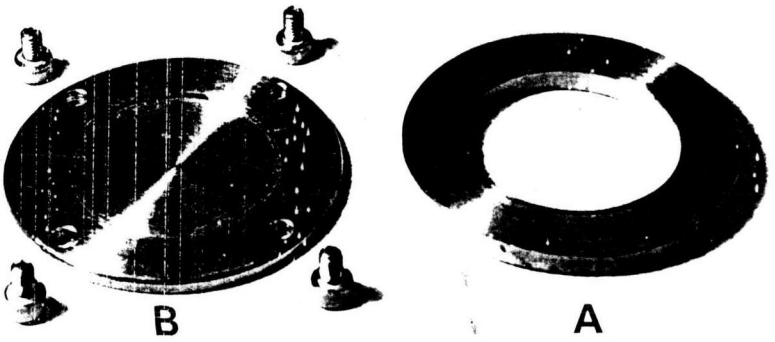
6.2 Determination of Drug Content in Preparations

The method for determining diclofenac content used in this study was modified from chemical characteristic of diclofenac and method for determining related drug (BP 1988). Only preparations that passed freeze-thaw cycle test with good appearance and no phase separation were accurately weighed about 2 g. into a 125 mL separator (separating funnel) which contained 10 mL of 0.1 N NaOH. The mixture was continously shaked for about 10 minutes, acidified with 2 mL of hydrochloric acid, and then extracted 3 times with 20 mL of chloroform, The chloroform phase was collected. Diclofenac was extracted from chloroform phase with 3 times of 20 mL of 0.1 N sodium hydroxide. The aqueous phase was collected into a 100 mL volumetric flask and adjusted to volume with 0.1 N sodium hydroxide. A 2 mL of aqueous phase was then diluted to 100 mL in a

100 mL volumetric flask with 0.1 N sodium hydroxide before analyzed spectrophotometrically at 276 nm using double beam spectrophotometer model 2000. A 0.1 N sodium hydroxide solution was used as a blank. Each sample were duplicately examined.

6.3 In-Vitro Release Studies

The selected formulations which passed the Freeze-Thaw cycle with no physical change and contained 100 + 10% lable amount (Babar et al.,1990; Rahman et al.,1990) were subjected to these studies. A dry compartment B of diffusion cell which shown in Figure 10 was accurately weighed. An approximate of 2 g. of preparation sample was placed into a compartment B 's chamber of 1.5 mm depth and 36 mm. diameter with an approximate surface area of 10 square centrimeters. The excess sample was removed with the edge of a spatula to proceduce an even, uniform sample surface. Compartment B with sample was accurately weighed and greased at the top surface around sample chamber with silicone vacuum grease. A hydrophobic Durapore$^{(R)}$ with 0.45 micron pore size and 45 mm diameter was completely covered over the sample. Compartment A was then joined together with compartment B and tightly screwed on. The sample-filled diffusion cell was then placed with compartment A and membrane upward, at the bottom and center of dissolution vessels which contained 250 mL of pH. 7.4 phosphate buffer, previously maintainedat 32 $\pm$ 0.5 ^{o}C.



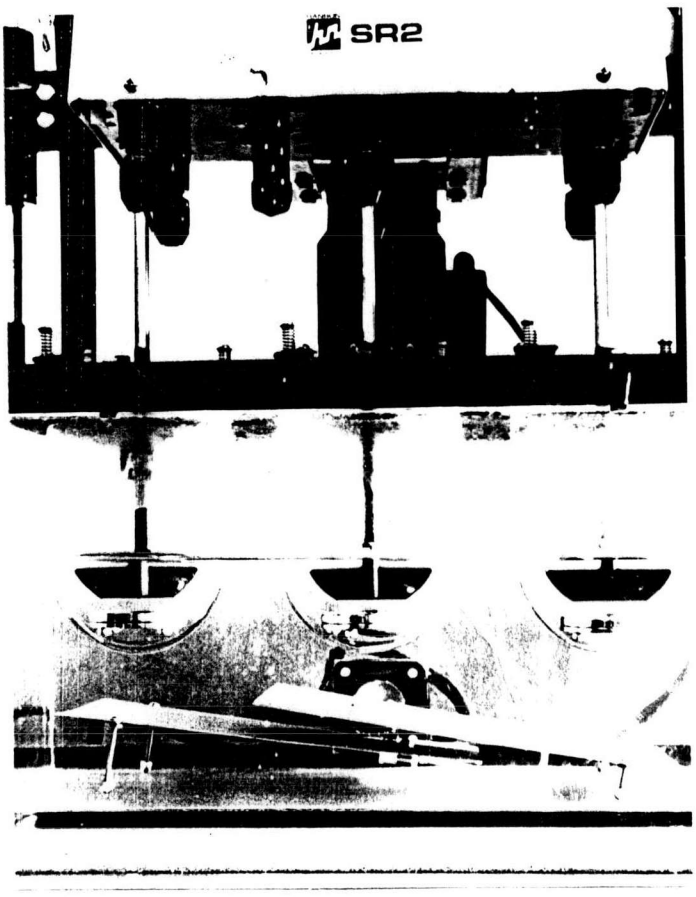


Figure 10 Schematic illustration of diffusion cell disk modified from disk of USP dissolution apparatus 3

Paddle of dissolution apparatus (Hanson Research Corporation, Model SR-2, USA) was adjusted at 2.5 cm above the membrane of diffusion cell and operated at the speed of 50 rpm. At predetermined time a 5 mL of diffusion medium was withdrawn for analysis of the amount of diclofenac diffused and replaced with an equal volume of pH 7.4 phosphate buffer to keep the diffusion medium volume constant. Each withdrawn sample was then diluted with methanol:water (1:1) to an optimum concentration. A diluted sample was analysed for their diclofenac content by HPLC. Each formulation was studied in triplicate.

7. High Performance Liquid Chromatography Methods

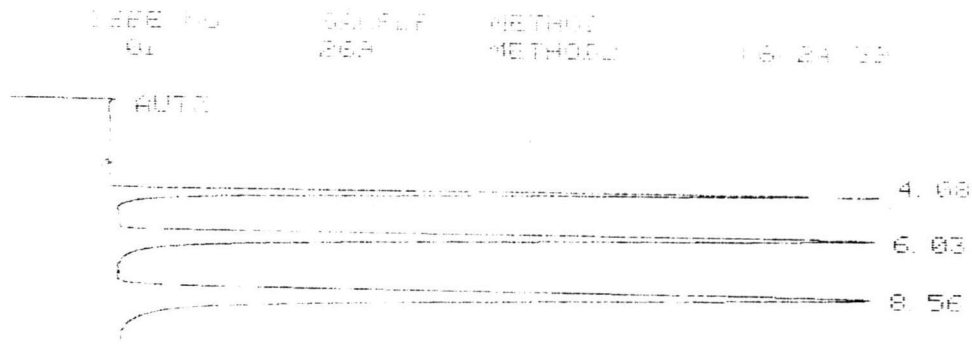
The analysis method for determination an amount of diclofenac with sensitive and specific is HPLC method. Peak of propyl paraben, diclofenac and mefenamic acid in HPLC chromatogram are separable. An HPLC chromatogram of propyl paraben, diclofenac and mefenamic acid was shown in Figure 11.

7.1 HPLC Method for Solubility and Stability of Diclofenac

High-performance liquid chromatographic condition for analysis of diclofenac solubility and stability was :

Column : micro-Bondapak$^{(R)}$ (Waters, USA)

Injector : 20 mcL



RUN TIME 12.00

DEFAULT 0
NORMALIZATION METHOD USING AREA

TIME	AREA	%AREA	PK NAME
4.08	1836621	21.97624568	
6.03	2766969	33.10763845	
8.56	3753711	44.91458426	
TOTAL	8357300	99.99846840	

Retention Time	Chemical reagents
4.08	Propyl paraben
6.03	Diclofenac
8.56	Mefenamic acid

Figure 11 HPLC chromatogram of propyl paraben, diclofenac and mefenamic acid

Eluent : methanol : water : glacial acetic acid (pH 3.5) = 70:30:1

Flow rate : 1.0 mL/min

Attenuation : 64

Solvent for dilution : methanol : water = 50:50

Detector : UV at 276 nm

7.1.1 Preparation of Internal Standard Stock Solution

Propyl paraben approximate 40 mg was accurately weighed and dissolved in solvent. The solution was then adjusted to 100 mL with solvent. A 25 mL of this solution was pipetted into a 100 mL volumetric flask and adjusted to volume with solvent. The final solution was used as internal standard stock solution.

7.1.2 Preparation of Sample Solution

A 2 mL of internal standard stock solution and optimum volume of sample were pipetted into 25 mL volumetric flask and altogether adjusted to volume with solvent.

7.1.3 Calibration Curve of Diclofenac

Diclofenac was accurately weighed, dissolved in solvent and diluted to optimun concentration. This

solution was used as a standard stock solution. Optimum volume of this solution was pipetted and adjusted to volume in 25 mL volumemetric flask which contained 2 mL of an internal standard stock solution in order to get appoximately 0 - 16 mcg/mL of diclofenac.

A HPLC chromatogram of calibration process of DS and DE was shown in Figure 12 and 14 ,respectively. A calibration curve data and calibration curve of DS and DE was shown in Table 9 and 10 and in Figure 13 and 15, respectively.

A calibration curve of diclofenac was done and calculated every time when a HPLC method was choosed for analysis.

7.2 HPLC Methods for Drug Diffusion Determination

High-performance liquid chromatographic condition for analysis of diclofenac diffusion was the same as 7.1 except the attenuation was changed from 64 to 4.

7.2.1 Preparation of Internal Standard Stock Solution

Mefenamic acid approximate 40 mg was accurately weighed and dissolved in methanol. The solution was then adjusted to 100 mL in a 100 mL

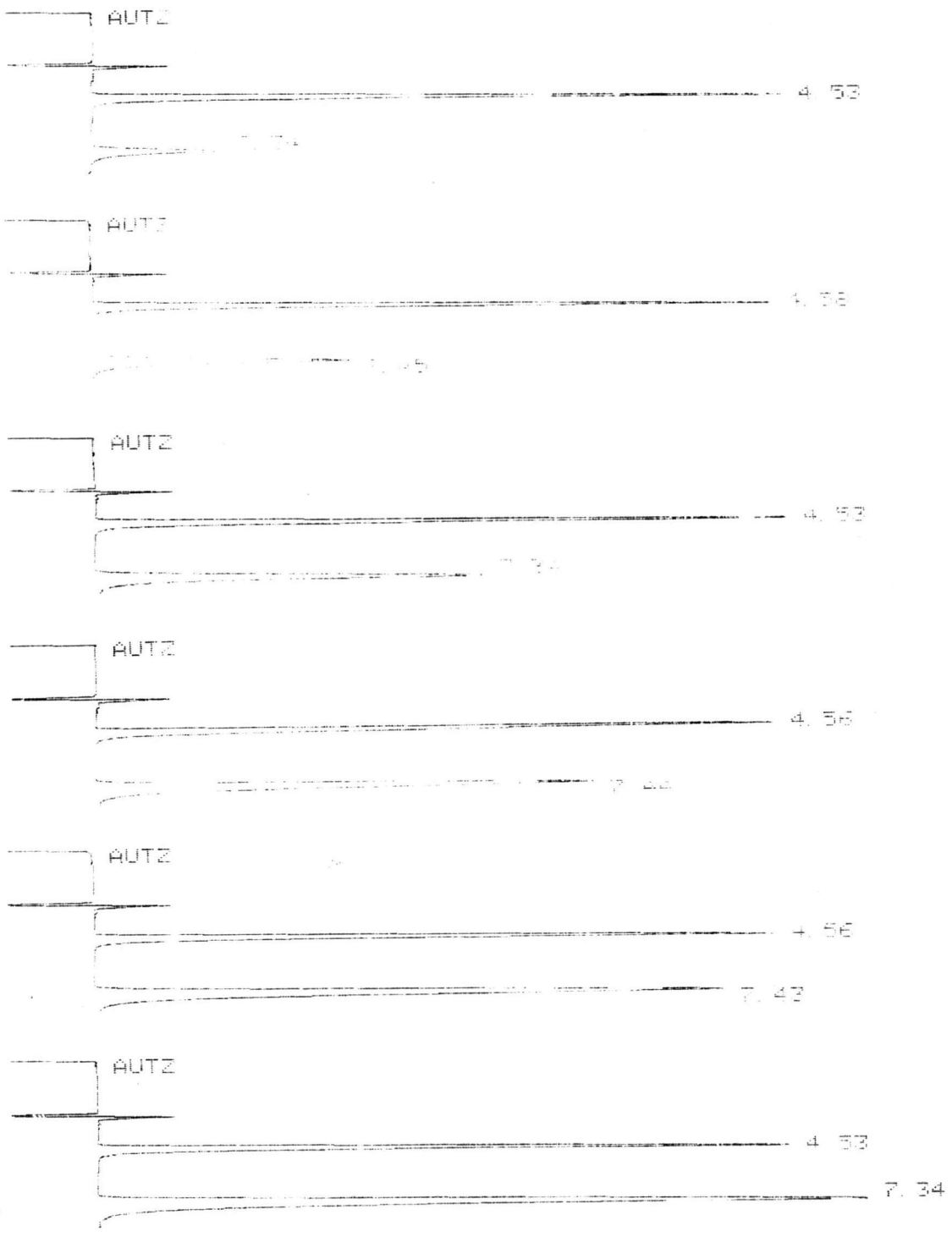


Figure 12 A HPLC chromatograms of DS and PP in standardization of solubility and stability study

Table 9 Calibration Data from HPLC Chromatogram of Diclofenac Sodium in Solubility and Stability study

Std	PP		DS		DS/PP ratio
	mcg/mL	Peak Area	mcg/mL	Peak Area	
1	8.780	764307	2.445	251358	0.328870
2	8.780	765017	4.890	501617	0.655694
3	8.780	770975	7.335	742206	0.962684
4	8.780	765977	9.780	988567	1.290597
5	8.780	766747	12.225	1232497	1.607437
6	8.780	765466	14.670	1473852	1.925429

X Coefficient	0.1310009
Constant	0.0063525
R Squared	0.9999445

n = 2

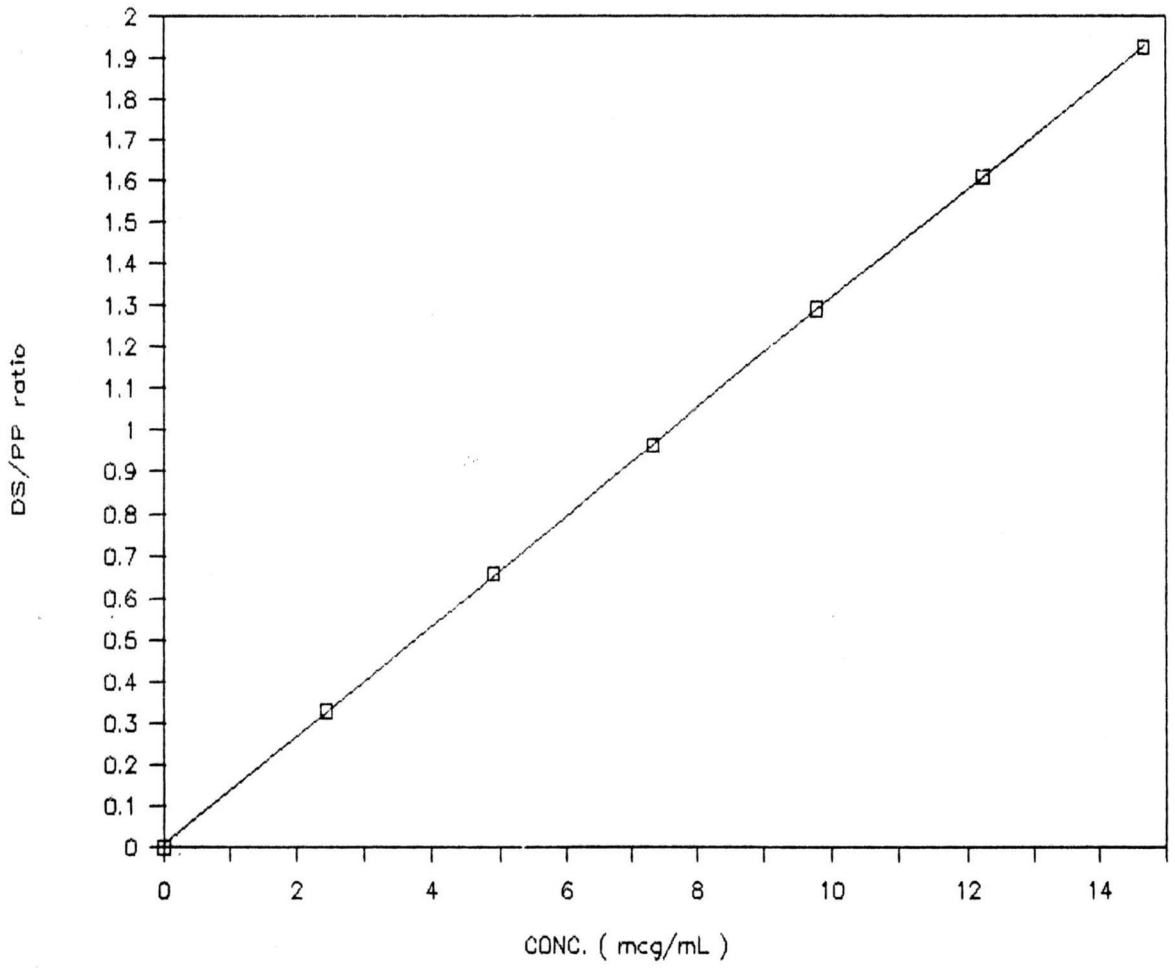


Figure 13 A HPLC calibration curve of DS solubility and stability study

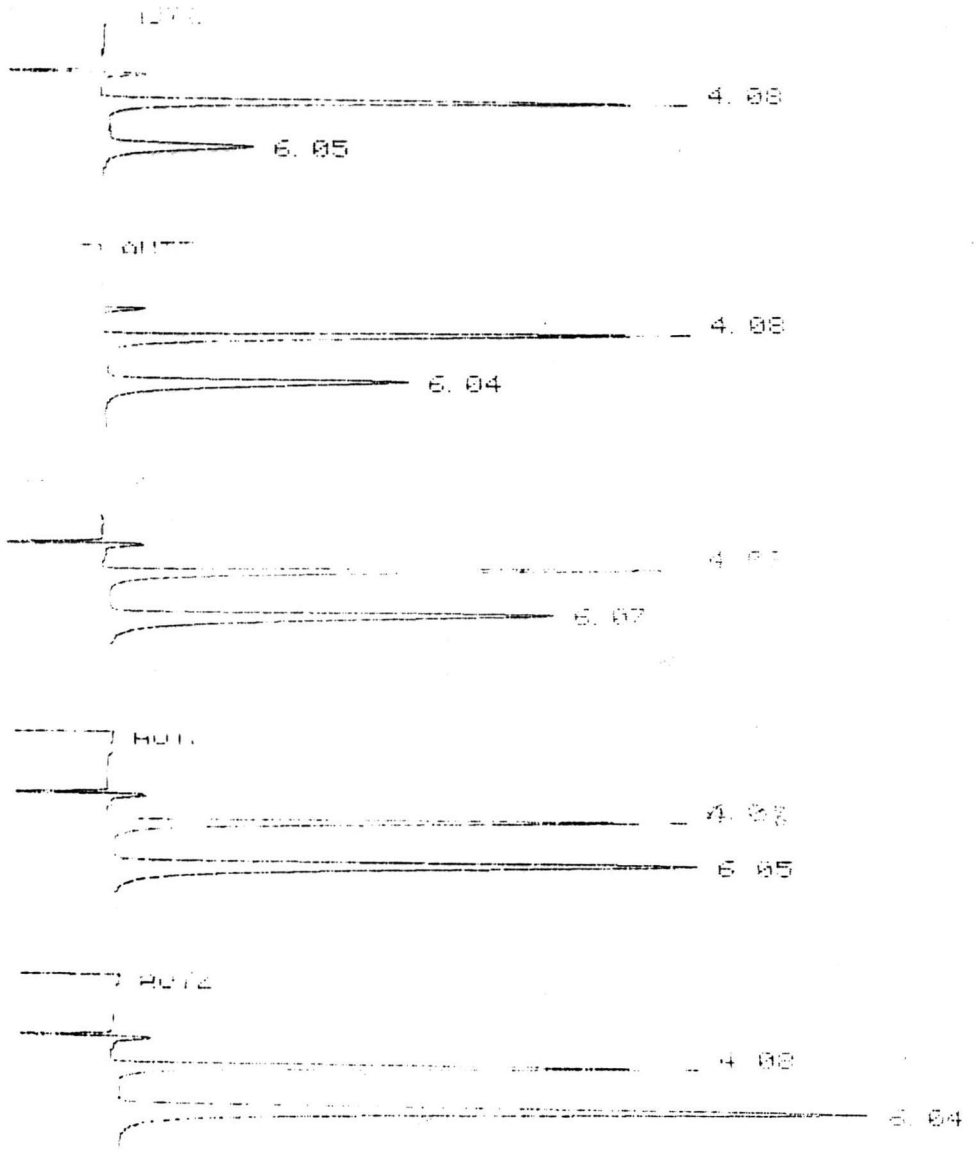


Figure 14 A HPLC chromatograms of DE and PP in standardization of solubility and stability study

Table 10 Calibration Data from HPLC Chromatogram of Diclofenac Diethylammonium in Solubility and Stability study

Std	PP		DE		DE/PP ratio
	mcg/mL	Peak Area	mcg/mL	Peak Area	
1	8.160	711735	3.304	300921	0.422798
2	8.160	708873	6.608	577389	0.814516
3	8.160	712970	9.912	867934	1.217350
4	8.160	711932	13.216	1144609	1.607751
5	8.160	711870	16.520	1433664	2.013942

X Coefficient	0.1213023
Constant	0.0107688
R Squared	0.9998920

n = 2

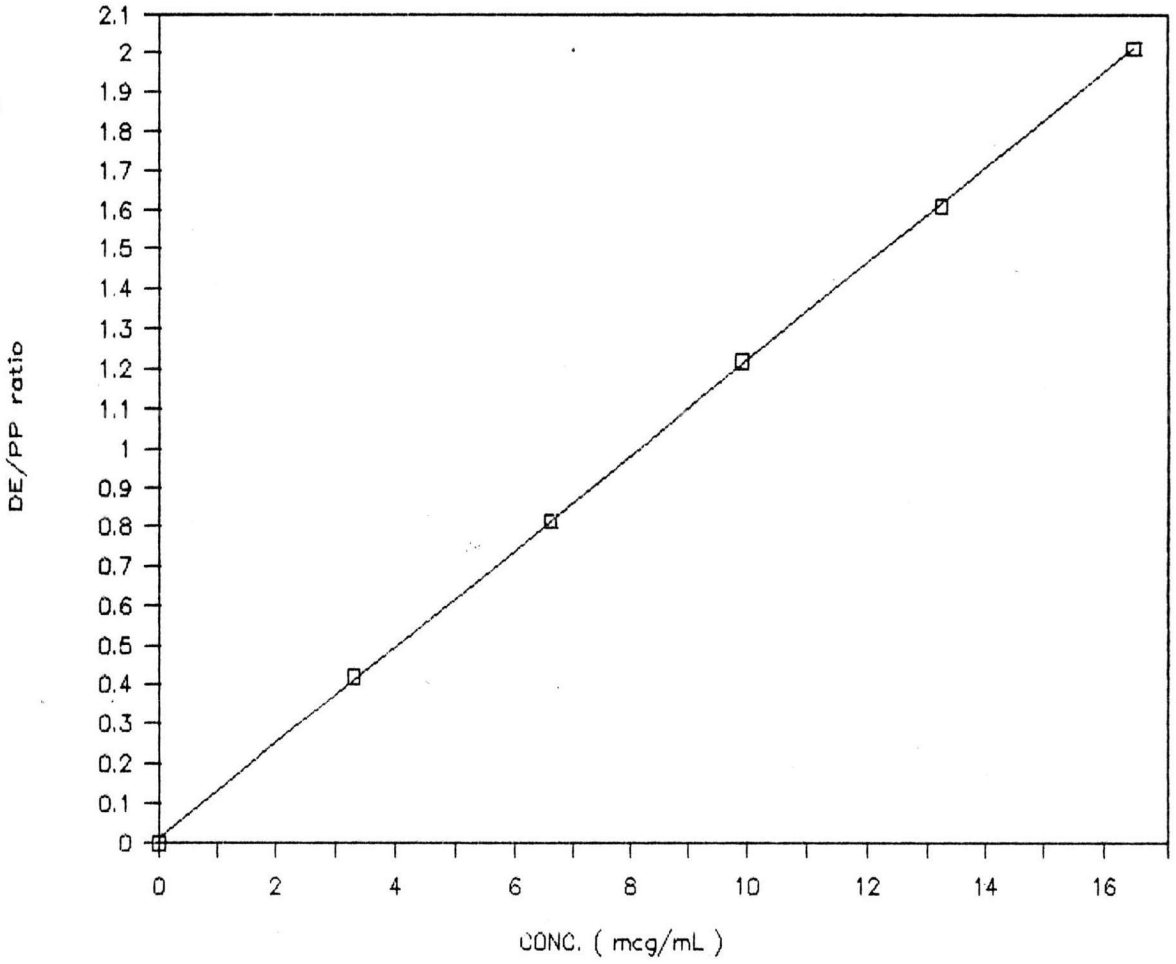


Figure 15 A HPLC calibration curve of DE solubility and stability study

volumetric flask with methanol. A 10 mL of solution was pipetted into a 25 mL volumetric flask and adjusted to volume with methanol. A 4 mL of diclofenac methanoic solution was pipetted into a 100 mL volumetric flask and adjusted to volume with solvent. This final solution was used as internal standard stock solution.

7.2.2 Preparation of Sample Solution

A 2 mL of internal standard stock solution and optimum volume of diffusion medium sample were pipetted into a 10 mL volumetric flask and altogether adjusted to volume with solvent.

7.2.3 Calibration Curve of DS and DE

Diclofenac was accurately weighed, dissolved in solvent and diluted to the desired concentration. This final solution was used as standard stock solution.

A 2 mL of internal standard stock solution and 1, 2, 3, 4 and 5 mL of standard stock solution were pipetted into a separately 10 mL volumetric flask and adjusted to volume with solvent. Finally, these solution were contained 0 - 1.3 mcg/mL of diclofenac.

A HPLC chromatogram of calibration process of DS and DE was shown in Figure 16 and 18 ,respectively. A

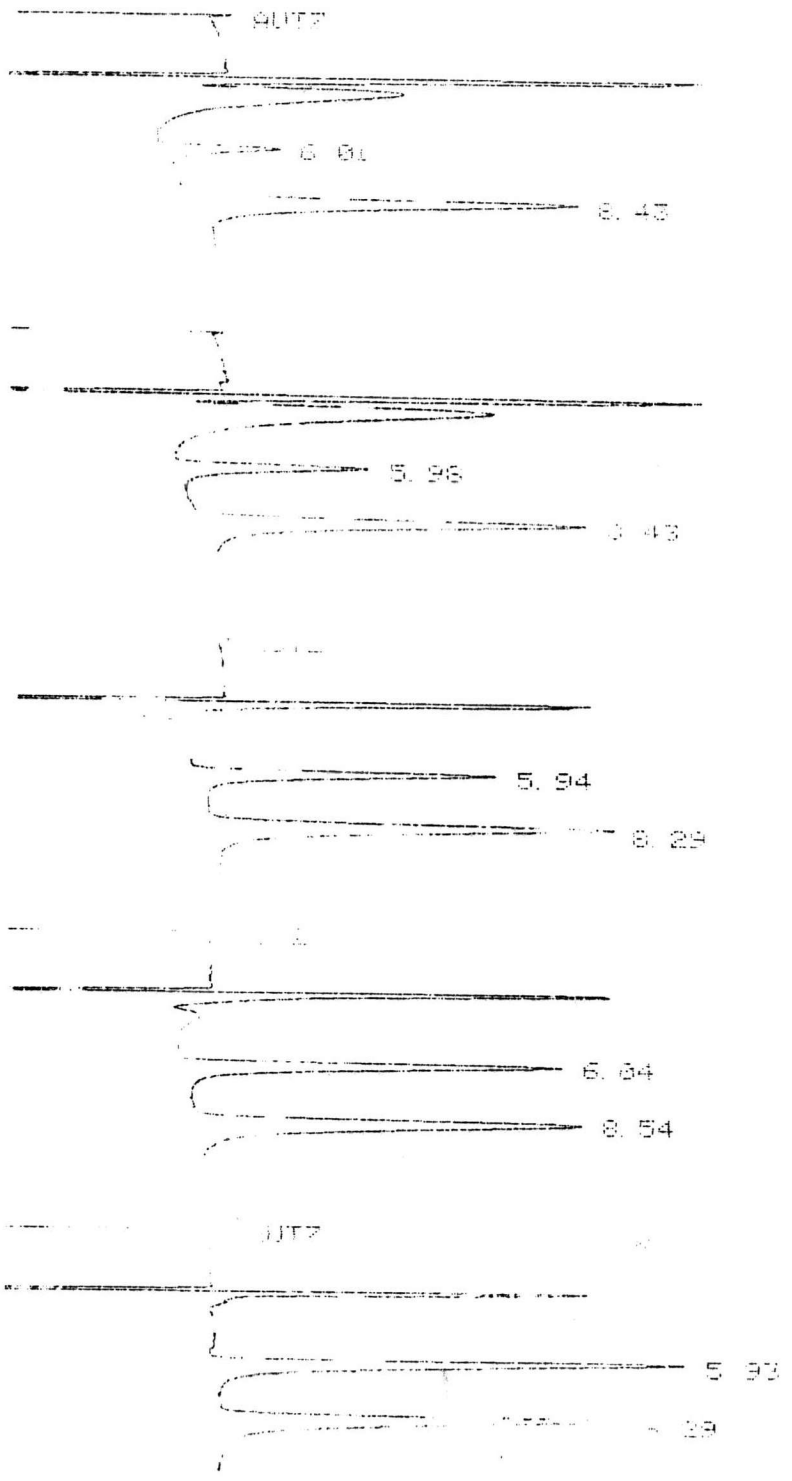


Figure 16 A HPLC chromatograms of DS and MFA in standardization of release study

Table 11 Calibration Data from HPLC Chromatogram of Diclofenac Sodium in Release study

Std	MFA		DS		DS/MFA ratio
	mcg/mL	Peak Area	mcg/mL	Peak Area	
1	1.395	108388	0.223	19380	0.178802
2	1.395	107043	0.446	38693	0.361471
3	1.395	107856	0.669	58350	0.540999
4	1.395	106979	0.892	77612	0.725488
5	1.395	108105	1.115	97051	0.897747

X Coefficient	0.808241
Constant	0.000156
R Squared	0.999937

n = 2

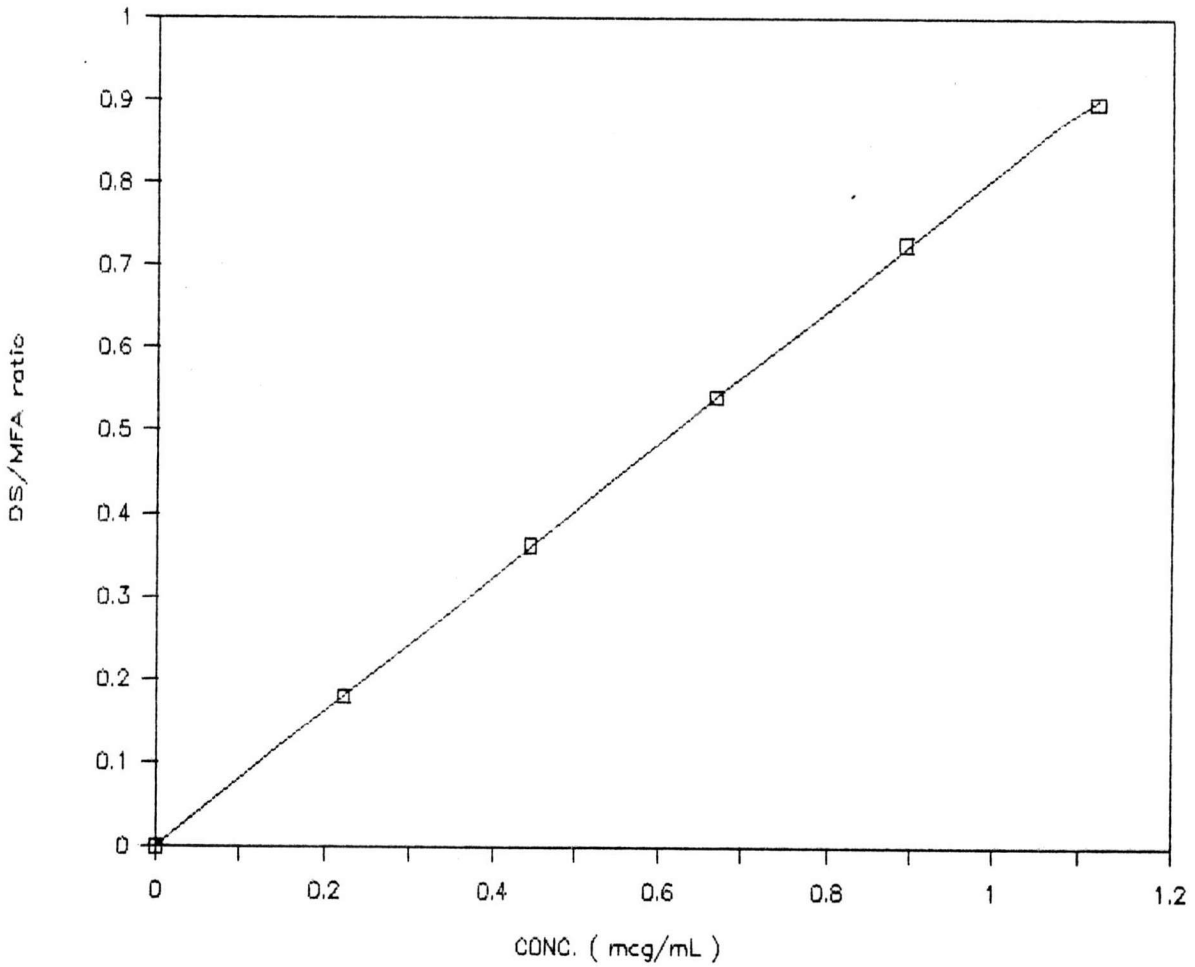


Figure 17 A HPLC calibration curve of DS release study

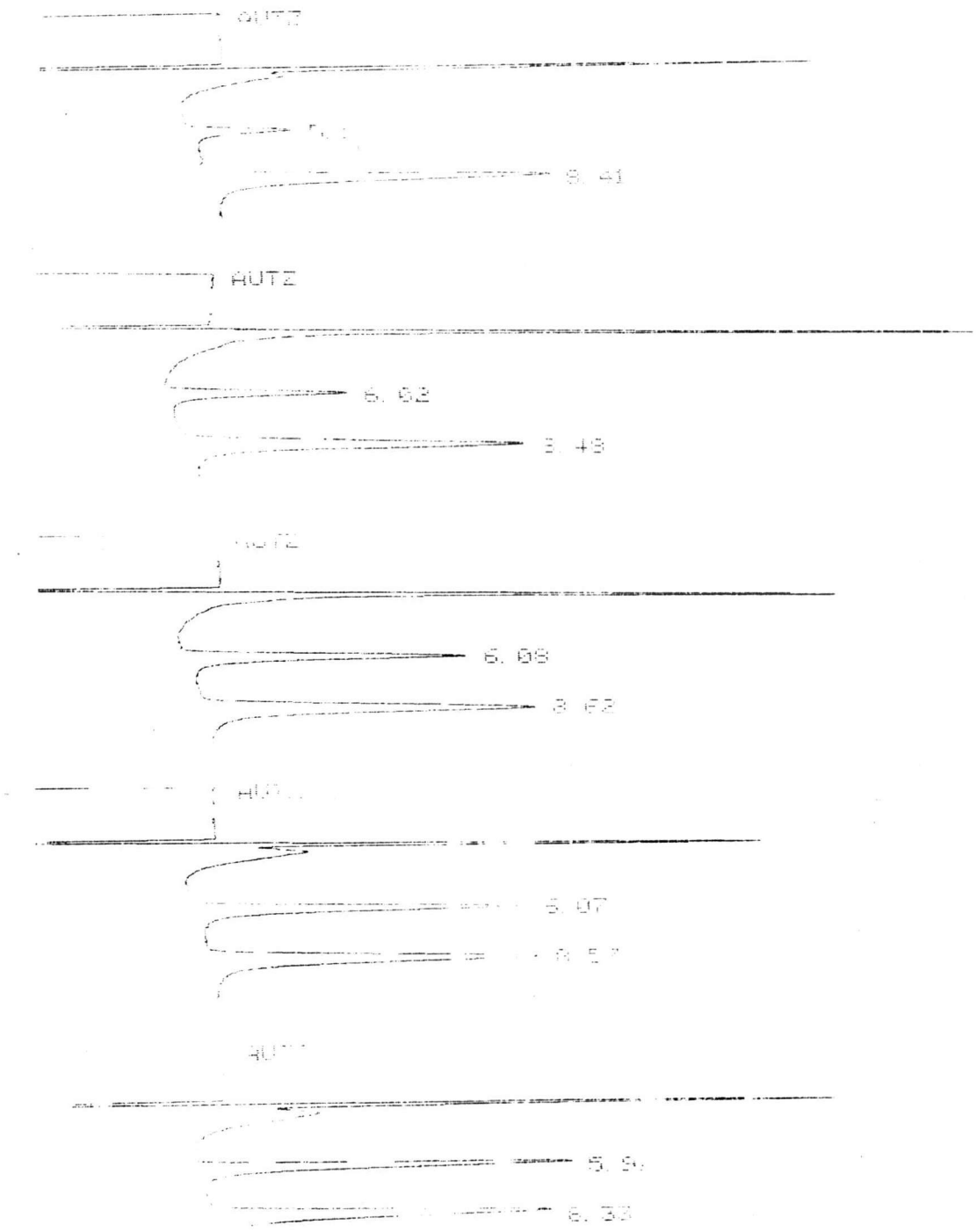


Figure 18 A HPLC chromatograms of DE and MFA in standardization of release study

Table 12 Calibration Data from HPLC Chromatogram of Diclofenac Diethylammonium in Release study

Std	MFA		DE		DE/MFA ratio
	mcg/mL	Peak Area	mcg/mL	Peak Area	
1	1.312	102015	0.260	19238	0.188580
2	1.312	100579	0.521	38820	0.385965
3	1.312	101680	0.781	58189	0.572275
4	1.312	100589	1.041	77590	0.771356
5	1.312	100413	1.302	97106	0.967066

X Coefficient	0.742981
Constant	-0.002690
R Squared	0.999923

n = 2

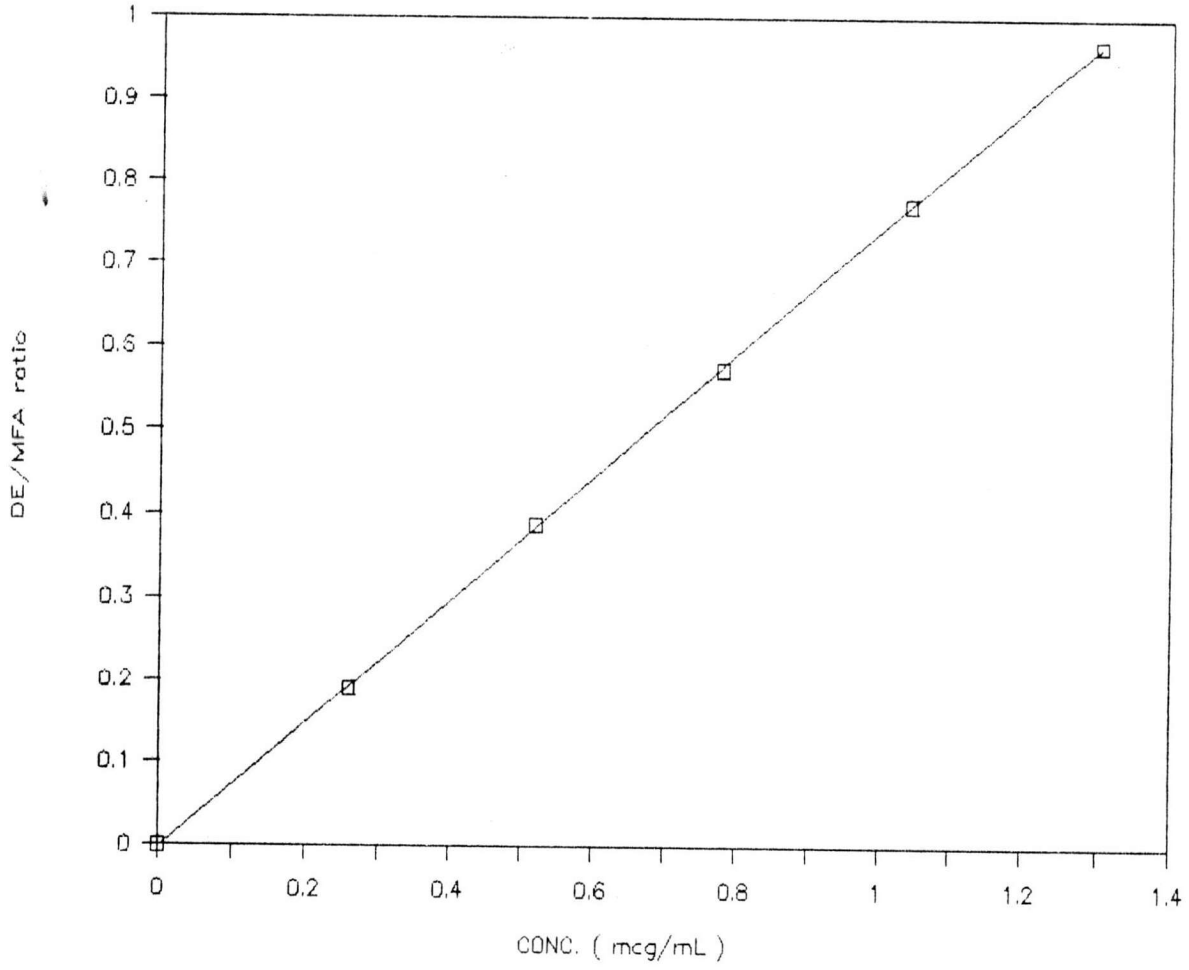


Figure 19 A HPLC calibration curve of DE release study

calibration curve data and calibration curve of DS and DE was shown in Table 11 and 12 and in Figure 17 and 19, respectively.

8. Calibration Curve of Diclofenac for Content Determination

Diclofenac 50 mg was accurately weighed and dissolved in 0.1 N sodium hydroxide solution. The solution was then adjusted to 50 mL with 0.1 N sodium hydroxide. A 5 mL of solution was pipetted into a 25 mL volumetric flask and adjusted to volume with 0.1 N sodium hydroxide. This final solution was used as stock solution. The stock solution was pipetted 1, 2, 3, 4 and 5 mL into separately 25 mL volumetric flask and diluted to volume with 0.1 N sodium hydroxide. The final drug concentration of each solution was 4, 8, 12, 16, 20 and 24 mcg/mL, respectively.

The absorbance of known drug concentration was determined by a double beam spectrophotometer in a 1-cm cell at 276 nm. The 0.1 N sodium hydroxide solution was used as a blank solution. Each concentration was determined in triplicate.

The concentration versus absorbance of diclofenac sodium and diclofenac diethylammonium presented in Table 13. , showed a linear relationship with correlation coefficients of 0.999982 and 0.999958 respectively. The

Table 13 UV Absorbance of DS and DE in 0.1N NaOH at Wavelenght of 276 nm

Concentration (mcg/mL)	Absorbance	
	DS	DE
0	0.000	0.000
4	0.125 [0.00057]	0.093 [0.001]
8	0.251 [0.00057]	0.184 [0.00057]
12	0.378 [0.00057]	0.273 [0.00057]
16	0.503 [0.00057]	0.362 [0.0]
20	0.626 [0.001]	0.452 [0.00057]
24	0.751 [0.00057]	0.544 [0.00057]

n = 3

standard curve of diclofenac after regression analysis was illustrated in Figure 20 , according to the equation A = 0.031312C + 0.000535 and A = 0.022571C + 0.001714, respectively. (Remark : A = absorbance, C = concentration)

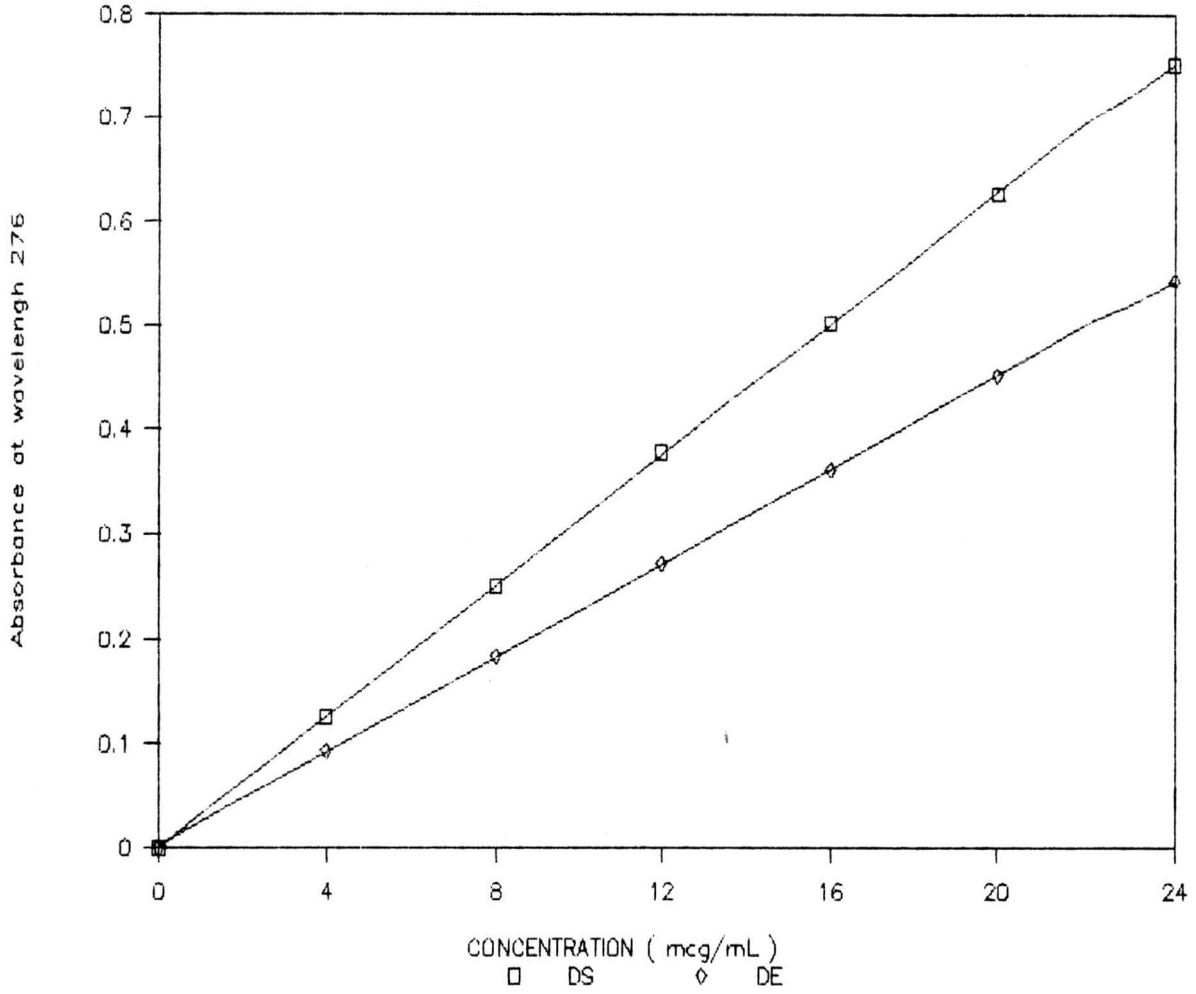


Figure 20 A UV spectrometrical calibration curve of DS and DE in 01 N NaOH solution at wavelenght of 276 nm