REFERENCES

1. Osol, A. and Pratt, R. in The United States Dispensary, 27th ed., pp. 750-751, J.B. Lippincott Company, Philadelphia, 1973.

2. Gilman, A.G., Goodman, L.S. and Gilman, A. in The Pharmacological Basis of Therapeutics, 6th ed., pp. 1448-1461, Macmillan Publishing, New York, 1980.

3. United States Pharmacopoeial Convention, Inc. "The United States Pharmacopoeia, 20th revision." pp. 526-527, Mack Printing Company, Easton, 1980.

4. British Pharmacopoeia Commission. "British Pharmacopoeia." pp. 291, 788, Her Majesty's Stationary Office, London, 1980.

5. Hosangadi, B.D. and Farias, C.M. "Determination of Methyltestosterone in Tablets" Indian Journal of Pharmaceutical Sciences 42(4),(1980): 124-125.

6. Carol, J. "The Determination of Methyltestosterone in Tablets by Absorption Spectrophotometry" Journal of the Association of Official Agricultural Chemists 34(1951): 572-576 through Chemical Abstracts 45(1951): 10494f.

7. Ito, A. and Amakasu, O. "Determination of Steroid Preparations by Infrared Spectrophotometry" Journal of the Pharmaceutical Society of Japan 77(1957):

1083-1086 through Chemical Abstracts 52(1957): 2666h.

8. Pawelczyk, E. and Opielewicz, M. "Gas Chromatography in Analysis of Drugs IV, Determination of Various Steroid Drugs" Farmacja Polska 34(9),(1978):531-534 through Analytical Abstracts 36(4),(1979): 4E20.

9. Bruschi, F. "Gas Chromatographic Determination of Mixtures of Tocopheryl Acetate and Methyltestosterone" Bollettino Chimico Farmaceutico 105(3),(1966): 237-240 through Analytical Abstracts 14(1967): 4982.

10. Jarzebinski, J., Baranowski, A., Hulpowska-Szulc, I. and Tonska, S. "Densitometric Determination of Active Components in Pharmaceutical Preparation V, Determination of Steroid Hormones" Acta Poloniae Pharmaceutica 36(4),(1979): 457-462.

11. Ivanova, N.M. and Sokolov, S.D. "Quantitative Determination of Steroid Hormones by Chromatospectrophotometry" Khim.-Farm. Zh. 15(6),(1981): 115-117 through Chemical Abstracts 95(1981): 103368s.

12. Huck, H. "Fluorescence of Oxidation Products of 3-oxo-Δ^4-Steroids on Alumina" Chromatographia 6(1), (1973): 46-49.

13. Wade, A. in Martindale The Extra Pharmacopoeia, 27th ed., p. 1408, Pharmaceutical Press, London, 1976-1977.

14. American Medical Association. "A M A Drug Evaluations." 4th ed., p. 658, A M A Department of Drugs, Illinois, 1980.

15. Oppenauer, R.V. "Dehydration of Secondary Alcohols to Ketones, I. Preparation of Sterol Ketones and Sex Hormones" Rec. Trav. Chim. 56(1937): 137-144 through Chemical Abstracts 31(1937): 3061[3].

16. Oppenauer, R. "Oxidation of Hormones." U.S. Pat 2,384, 335 Sept. 4, 1945 through Chemical Abstracts 40(1946): 178-179.

17. Miescher, K. and Wettstein, A. "Saturated and Unsaturated 17-Hydroxyandrostanes." U.S. Pat 2,374,370 April 24, 1945 through Chemical Abstracts 39(1945): 5412-5413.

18. Miescher, K. "17-Substituted Testosterones." U.S. Pat 2,386,331 Oct. 9, 1945 through Chemical Abstracts 40(1946): 178.

19. Miescher, K. "3-Thio Ethers of Androstanolones." U.S. Pat 2,435,013 Jan. 27, 1948 through Chemical Abstracts 42(1948): 2996c.

20. Wilson, C.O., Gisvold, O. and Doerge, R.F. in Textbook of Organic Medicinal and Pharmaceutical Chemistry, 6th ed., p. 801, J.B. Lippincott Company, Philadelphia, 1971.

21. Osol, A. in Remington's Pharmaceutical Sciences, 16th ed., p. 941, Mack Publishing Company, Easton, 1980.

22. Windholz, M. et al. in The Merck Index, 9th ed., p. 799, Merck & Co., Inc., New Jersy, 1976.

23. Abelson, D.M. and Bondy, P.K. "Fluorometric Determination of Δ^4-3-Ketosteroids" Biochimica et Biophysica Acta 57(1955): 208-217.

24. Abelson, D.M., Bondy, P.K. and Piskorski, J. "Determination of Steroids by Paper Strip Elution Chromatography" Journal of Chromatography 5(1961): 332-335.

25. Bush, I.E. "Methods of Paper Chromatography of Steroids Applicable to the Study of Steroids in Mammalian Blood and Tissues" The Biochemical Journal 50 (1952): 370-377.

26. Bush, I.E. and Sandberg, A.A. "Adrenocortical Hormones in Human Plasma" J. Biol. Chem. 205(1953): 783-793.

27. Egg, D. and Huck, H. "New Fluorimetric Determination of Urinary Testosterone by Thin-Layer Densitometry" Journal of Chromatography 63(1971): 349-355.

28. Egg, D. "Determination of Progesterone in Plasma by Thin-Layer Densitometry" Journal of Chromatography 86(1973): 151-157.

29. Sittisomwong, N. "Determination of Contraceptive Hormones Available in Thailand" Report, Drug Analysis Division, Department of Medical Sciences, Bangkok, 1978.

30. Bethke, H., Santi, W. and Frei, R.W. "Data-Pair Tech-

nique, a New Approach to Quantitative Thin-Layer Chromatography" Journal of Chromatographic Sciences 12(1974): 392-397.

APPENDIX

Table 1. Thin- Layer Chromatography of Methyltestosterone

Mobile phase	Ratio	R_f value	Shape of spot	Separation
Dichloromethane	-	0.46	round	overlap
Dichloromethane-hexane	80 : 20	0.26	round	overlap
	90 : 10	0.28	round	overlap
	95 : 5	0.30	round	overlap
Benzene-absolute ethanol	95 : 5	0.86	elongated	overlap
	99 : 1	0.80	round	overlap
Benzene-diethyl ether	40 : 60	0.33	elongated	completely separated
	50 : 50	0.26	elongated	completely separated
	60 : 40	0.21	round	completely separated
	70 : 30	0.18	round	completely separated
	90 : 10	0.10	round	completely separated

Sample size 1 ul of methyltestosterone 0.1 mg/ml

Table 2. Effect of Time on Fluorescent Spot Intensity of Methyltestosterone

Time (minutes)	Peak height[a] (mm)
0*	72.7
30	72.7
60	72.3
90	71.0
240	69.0
270	68.3
300	68.0
24 (hours)	60.5

Sample size 1 µl of methyltestosterone 0.1 mg/ml
Layer 0.25 mm aluminum oxide F_{254} (type T)
Mobile phase benzene-diethyl ether 60:40
Detection 20 min., 150°, fluorescence measurement, $\lambda_{ex.}$ 366 nm, $\lambda_{em.}$ 454 nm

Time " 0* " means the fluorescence intensities was measured after keeping in a desiccator for 1 hour.

[a] Mean value of three measurements.

Table 3. A Comparison of the Reproducibility of Fluorescence Measurements by a Single Value and a Data-Pair Approach

Single value		Data-pair	
Spot no.	Peak height(mm)	Spot no.	Peak height(mm)
1	71.6	1+6	74.0
2	74.8	2+7	76.4
3	75.7	3+8	76.1
4	75.9	4+9	75.8
5	79.1	5+10	76.9
6	76.3		
7	78.0		
8	76.5		
9	75.7		
10	74.7		
Mean	75.8		75.8
Coeff. of var.	2.65 %		1.46 %

Sample size 1 μl of methyltestosterone 0.1 mg/ml

Layer 0.25 mm aluminum oxide F_{254} (type T)

Mobile phase benzene-diethyl ether 60:40

Detection 20 min., 150°, fluorescence measurement, $\lambda_{ex.}$ 366 nm, $\lambda_{em.}$ 454 nm

Table 4. Relationship between Concentration and Peak Height of Methyltestosterone

Concentration (ng)	Peak height[a] (mm)
25	15.7
75	46.1
100	60.2
125	74.4
150	88.2
200	116.2
225	127.5
250	133.7

Sample size 1 µl of methyltestosterone solutions
Layer 0.25 mm aluminum oxide F_{254} (type T)
Mobile phase benzene-diethyl ether 60:40
Detection 20 min., 150°, fluorescence measurement, $\lambda_{ex.}$ 366 nm, $\lambda_{em.}$ 454 nm

[a] Mean value of three measurements.

Table 5. A Comparison of R_f Values and Peak Heights between Methyltestosterone Spots with and without Ethinyl Estradiol

Spot of	R_f value[a]	Peak height[a](mm)
Methyltestosterone	0.22	62.0
Mixture of methyltesto-sterone and ethinyl estradiol	0.22	62.0
Ethinyl estradiol	cannot be detected	-

Sample size 1 µl of methyltestosterone 0.1 mg/ml
Layer 0.25 mm aluminum oxide F_{254} (type T)
Mobile phase benzene-diethyl ether 60:40
Detection 20 min., 150°, fluorescence
(a) in UV-light (366 nm)
(b) measurement, $\lambda_{ex.}$ 366 nm, $\lambda_{em.}$ 454 nm

[a] Mean value of two spots.

Table 6. A Comparison of R_f Values and Peak Heights between Methyltestosterone Spots with and without Fat-Soluble Vitamins

Spot of	R_f value[a]	Peak height[a](mm)
Methyltestosterone	0.22	62.0
Mixture of methyltesto-sterone and fat-soluble vitamins	0.22	61.3
Fat-soluble vitamins	0.92	-

Sample size 1 µl of methyltestosterone 0.1 mg/ml
Layer 0.25 mm aluminum oxide F_{254} (type T)
Mobile phase benzene-diethyl ether 60:40
Detection 20 min., 150°, fluorescence
(a) in UV-light (366 nm)
(b) measurement, $\lambda_{ex.}$ 366 nm, $\lambda_{em.}$ 454 nm

[a] Mean value of two spots.

Table 7. A Comparison of R_f Values and Peak Heights between Methyltestosterone Spots with and without Water-Soluble Vitamins

Spot of	R_f value[a]	Peak height[a](mm)
Methyltestosterone	0.21	73.6
Mixture of methyltesto-sterone and water-soluble vitamins	0.21	74.5
Water-soluble vitamins	0	-

Sample size 1 µl of methyltestosterone 0.1 mg/ml
Layer 0.25 mm aluminum oxide F_{254} (type T)
Mobile phase benzene-diethyl ether 60:40
Detection 20 min., 150°, fluorescence
(a) in UV-light (366 nm)
(b) measurement, $\lambda_{ex.}$ 366 nm, $\lambda_{em.}$ 454 nm

[a] Mean value of two spots.

Table 8. A Comparison of R_f Values and Peak Heights between Methyltestosterone Spots with and without Minerals

Spot of	R_f value[a]	Peak height[a](mm)
Methyltestosterone	0.26	94.0
Mixture of methyltesto-sterone and minerals	0.26	94.5
Minerals	cannot be detected	-

Sample size 1 µl of methyltestosterone 0.1 mg/ml
Layer 0.25 mm aluminum oxide F_{254} (type T)
Mobile phase benzene-diethyl ether 60:40
Detection 20 min., 150°, fluorescence
(a) in UV-light (366 nm)
(b) measurement, $\lambda_{ex.}$ 366 nm, $\lambda_{em.}$ 454 nm

[a] Mean value of two spots.

Table 9. A Comparison of R_f Values and Peak Heights between Methyltestosterone Spots with and without Other Compounds

Spot of	R_f value[a]	Peak height[a](mm)
Methyltestosterone	0.21	73.0
Mixture of methyltesto-sterone and other compounds	0.21	70.3
Other compounds	0	-

Sample size 1 µl of methyltestosterone 0.1 mg/ml
Layer 0.25 mm aluminum oxide F_{254} (type T)
Mobile phase benzene-diethyl ether 60:40
Detection 20 min., 150°, fluorescence
(a) in UV-light (366 nm)
(b) measurement, $\lambda_{ex.}$ 366 nm, $\lambda_{em.}$ 454 nm

[a] Mean value of two spots.

Table 10. A Comparison of R_f Values and Peak Heights between Methyltestosterone Spots with and without Vegetable Oil

Spot of	R_f value[a]	Peak height[a](mm)
Methyltestosterone	0.21	72.7
Mixture of methyltesto-sterone and cottonseed oil	0.21	70.3
Cottonseed oil	0.87	-

Sample size 1 µl of methyltestosterone 0.1 mg/ml
Layer 0.25 mm aluminum oxide F_{254} (type T)
Mobile phase benzene-diethyl ether 60:40
Detection 20 min., 150°, fluorescence
(a) in UV-light (366 nm)
(b) measurement, $\lambda_{ex.}$ 366 nm, $\lambda_{em.}$ 454 nm

[a] Mean value of two spots.

Table 11. Percent Labelled Amount of Methyltestosterone in Methyltestosterone Tablet Using Spectrofluorodensitometric Method and USP Method

Sample	Percent Labelled Amount of Methyltestosterone	
	Spectrofluorodensitometric method	USP method
1	91.96	92.44
2	93.28	91.68
3	91.84	93.60
4	92.44	92.24
5	91.44	92.84
Mean	92.20	92.56
Coeff. of var.(%)	0.76	0.78

Table 12. Percent Labelled Amount of Methyltestosterone in Vitamin-Hormone Preparations Using Spectrofluorodensitometric Method

Sample	Label content (mg)	Amount found[a] (mg)	% Labelled amount	% CV
1	0.8	0.81	101.25	1.98
2	1.0	0.99	99.00	2.20
3	1.0	0.99	99.00	3.81
4	2.0	1.90	95.00	1.89
5	2.5	2.34	93.60	2.20

[a] Mean value of three experiments.

Table 13. Percent Recovery of Methyltestosterone in Vitamin-Hormone Preparation

Sample	Amount added (mg)	Amount found (mg)	% Recovery
1	0.50	0.490	98.00
2	0.50	0.508	101.60
3	0.50	0.498	99.60
Mean			99.73
Coeff. of var. (%)			1.80
1	1.00	1.040	104.00
2	1.00	0.990	99.00
3	1.00	0.996	99.60
Mean			100.87
Coeff. of var. (%)			2.71
1	1.50	1.480	98.67
2	1.50	1.520	101.33
3	1.50	1.474	98.27
Mean			99.42
Coeff. of var. (%)			1.67

Table 14. Analysis of Vitamin - Hormone Preparations Containing Methyltestosterone

Sample	Formula	Label content (mg)	Amount[a] found (mg)	% Labelled amount	% CV
1	A	0.8	0.81	101.25	1.98
2	B	1.0	0.99	99.00	2.20
3	B	1.0	0.99	99.0	3.81
4	B	2.0	2.13	106.50	2.99
5	B	2.0	1.90	95.00	1.89
6	B	2.0	1.88	94.00	3.84
7	B	2.5	2.34	93.60	2.56
8	B	2.5	2.34	93.60	2.35
9	B	4.0	3.65	91.25	1.52
10	B	20.0	20.09	100.45	1.23
11	C	5.0	4.59	91.80	3.05

A Methyltestosterone combined with other hormone and vitamins.

B Methyltestosterone combined with other hormone, vitamins and minerals.

C Methyltestosterone combined with vitamins and alkaloids, strychnine and yohimbine.

a Mean value of three experiments.

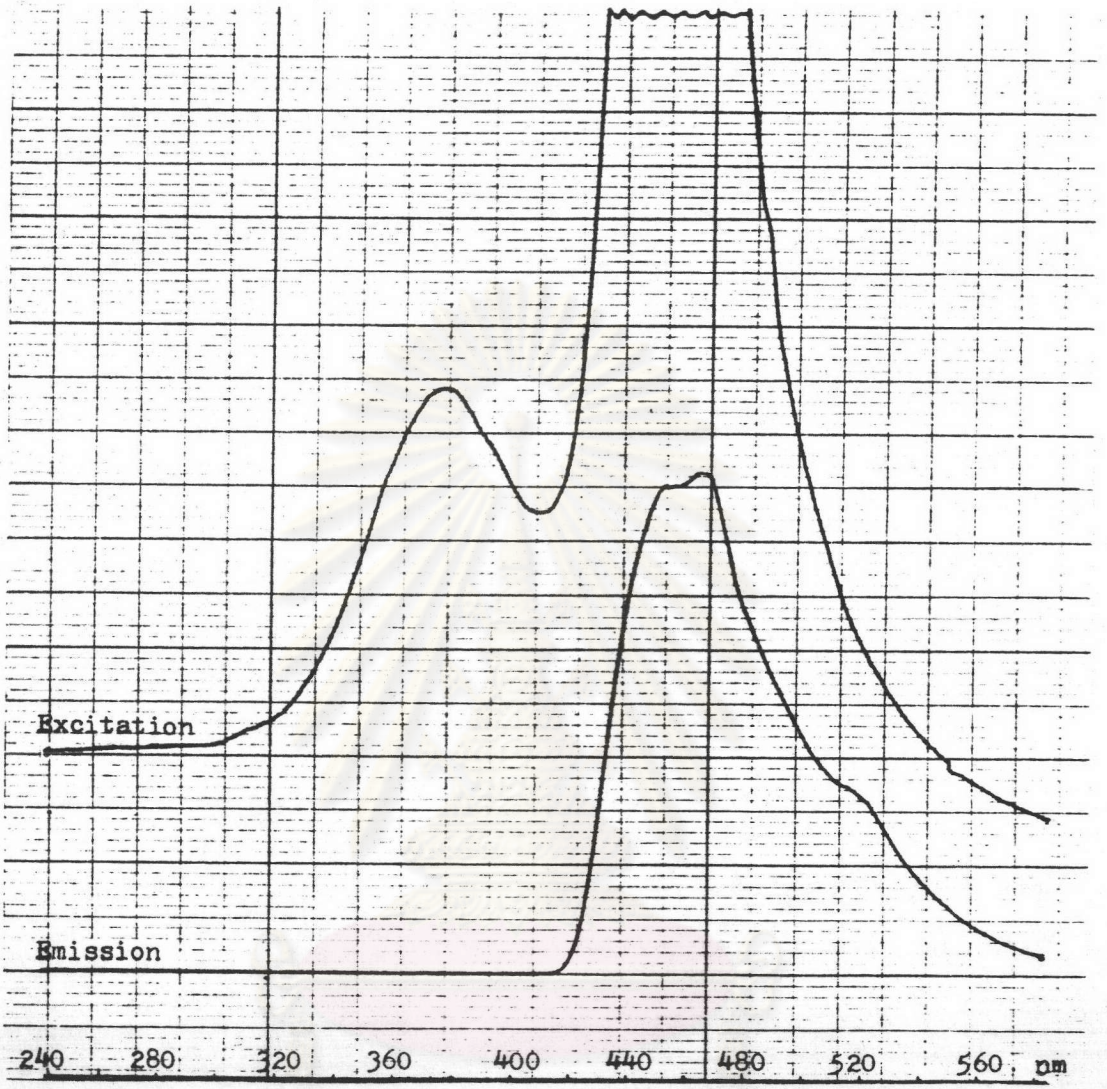

Figure 1. Fluorescence Spectra of Methyltestosterone
Excitation spectrum with emission at 454 nm;
emission spectrum with excitation at 366 nm.

Layer 0.25 mm aluminum oxide F_{254} (type T)
Mobile phase benzene-diethyl ether 60:40
Detection 20 min., 150°, measured with a fluorescence spectrophotometer Perkin Elmer MPF-3 connection with thin-layer accessories.

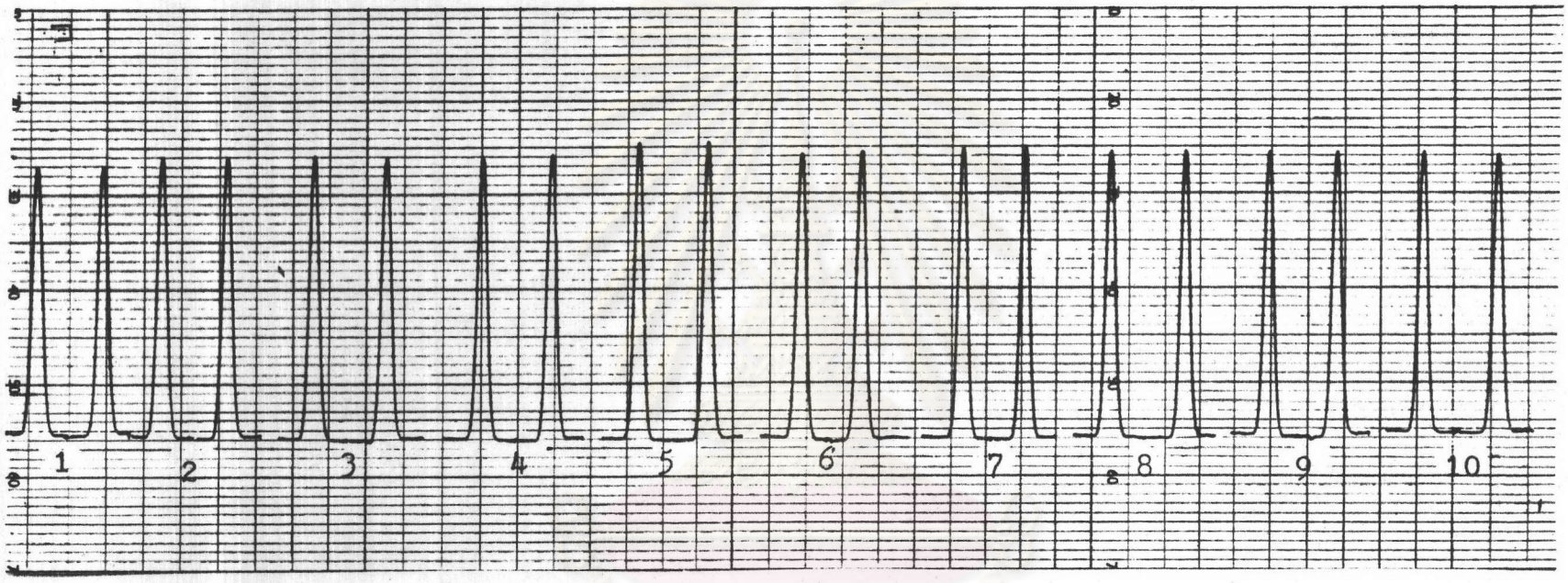

Figure 2. Chromatogram Fluorescence Intensity of Methyltestosterone Spots within One Plate

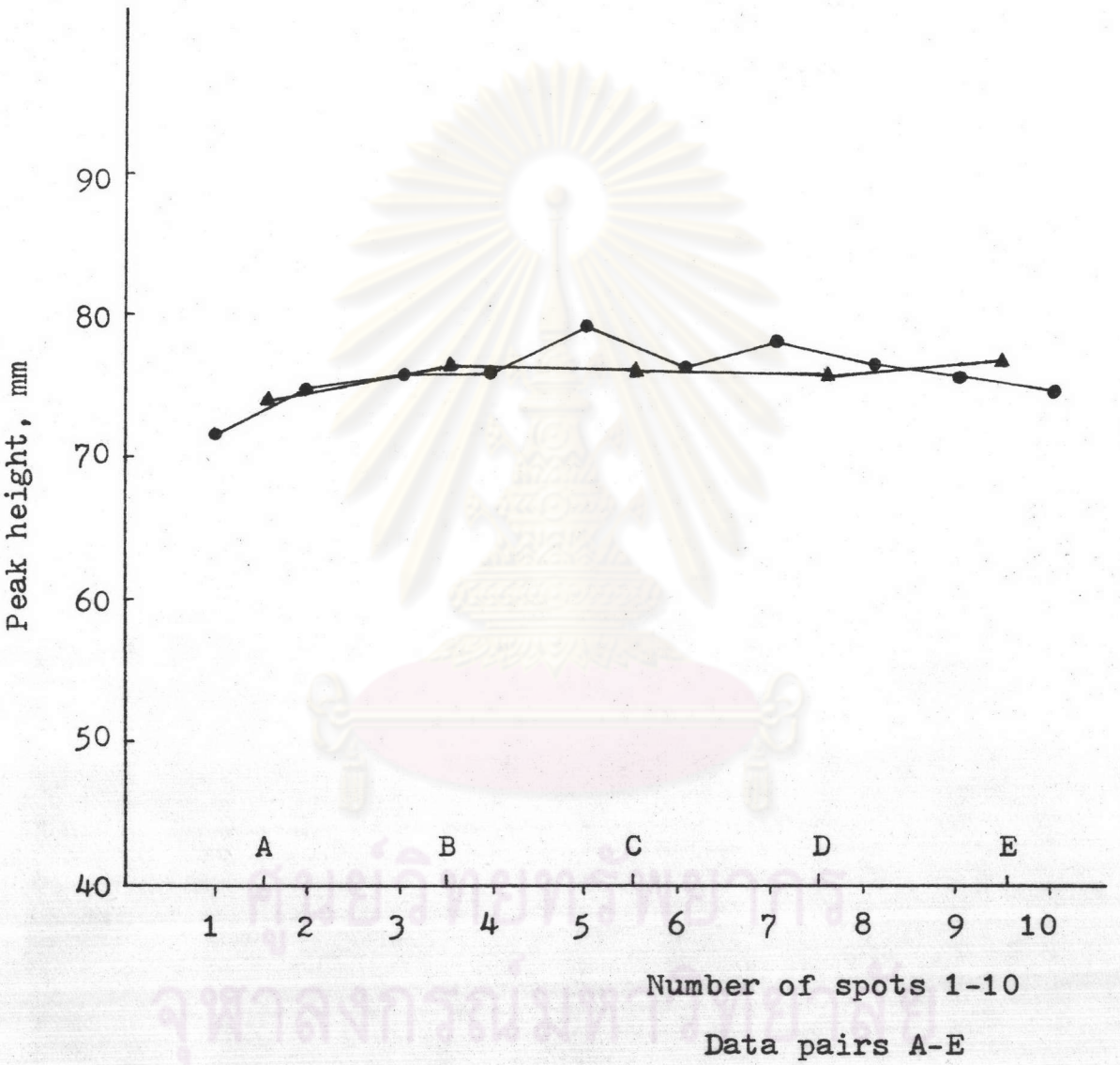

Figure 3. A Comparison of R_f Patterns for Methyltestosterone Spots

▲—▲ data-pair values

●—● single values

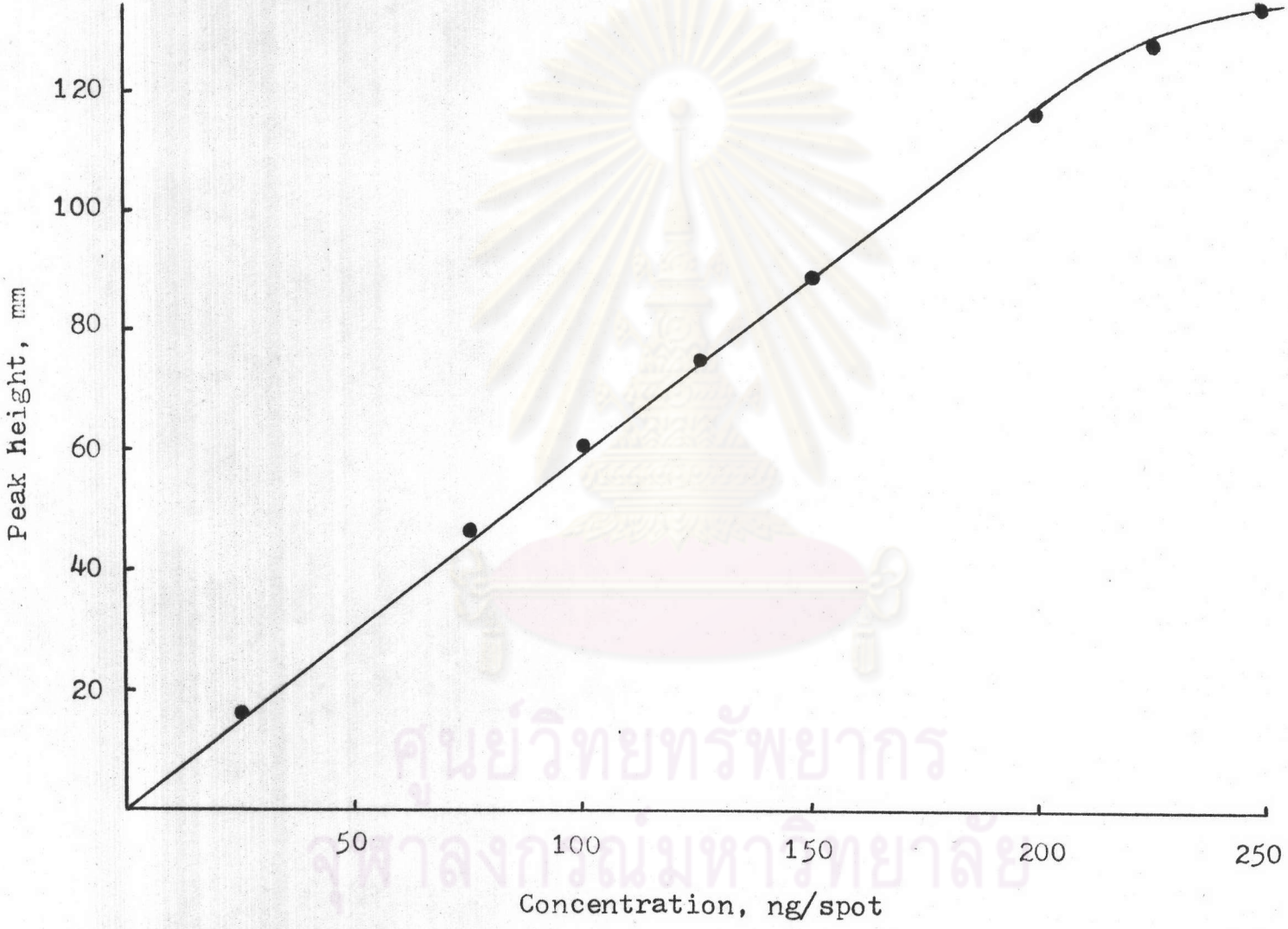

Figure 4. Calibration Curve of Methyltestosterone

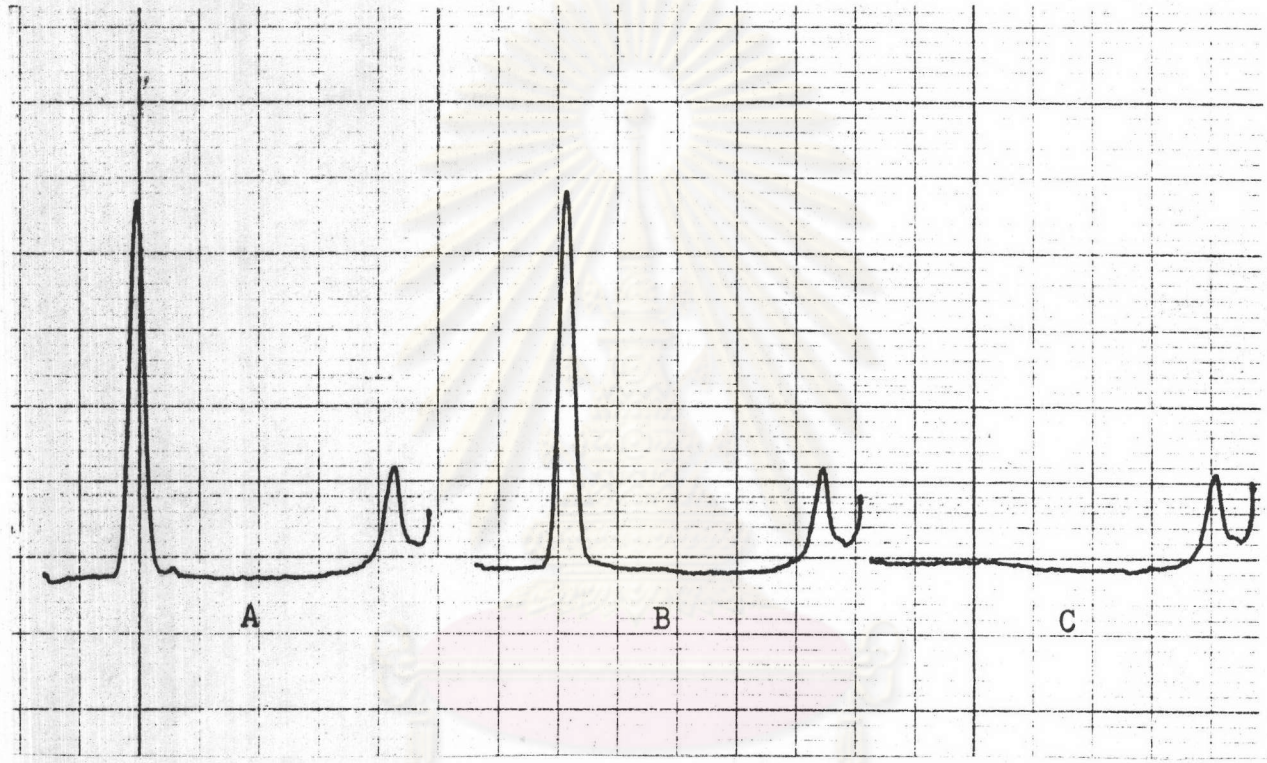

Figure 5. Chromatogram Fluorescence Intensity of

A. Methyltestosterone

B. Mixture of methyltestosterone and ethinyl estradiol

C. Ethinyl estradiol

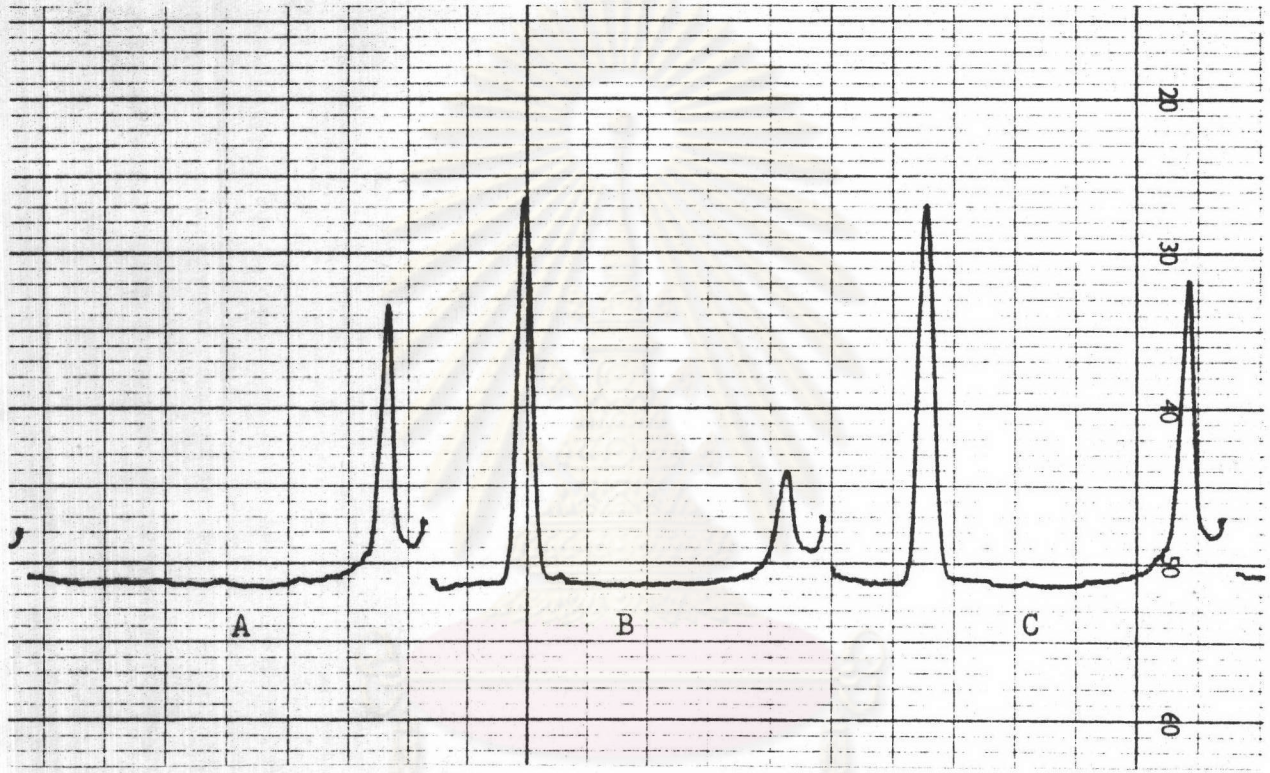

Figure 6. Chromatogram Fluorescence Intensity of

A. Fat-soluble vitamins

B. Methyltestosterone

C. Mixture of methyltestosterone and fat-soluble vitamins

Figure 7. Chromatogram Fluorescence Intensity of

A. Methyltestosterone

B. Mixture of methyltestosterone and water-soluble vitamins

C. Water-soluble vitamins

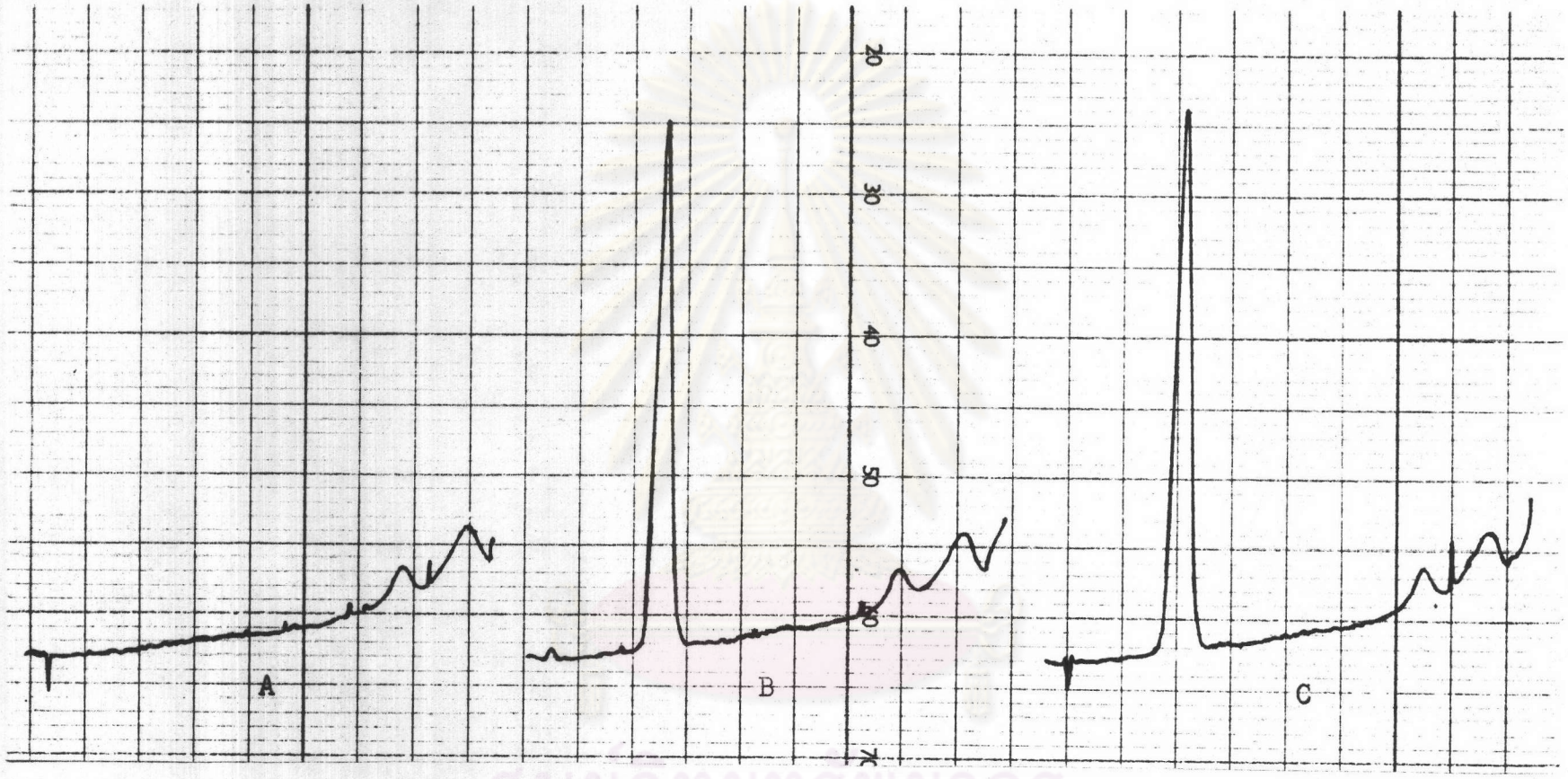

Figure 8. Chromatogram Fluorescence Intensity of

A. Minerals

B. Methyltestosterone

C. Mixture of methyltestosterone and minerals

Figure 9. Chromatogram Fluorescence Intensity of

A. Methyltestosterone

B. Mixture of methyltestosterone and other compounds

C. Other compounds

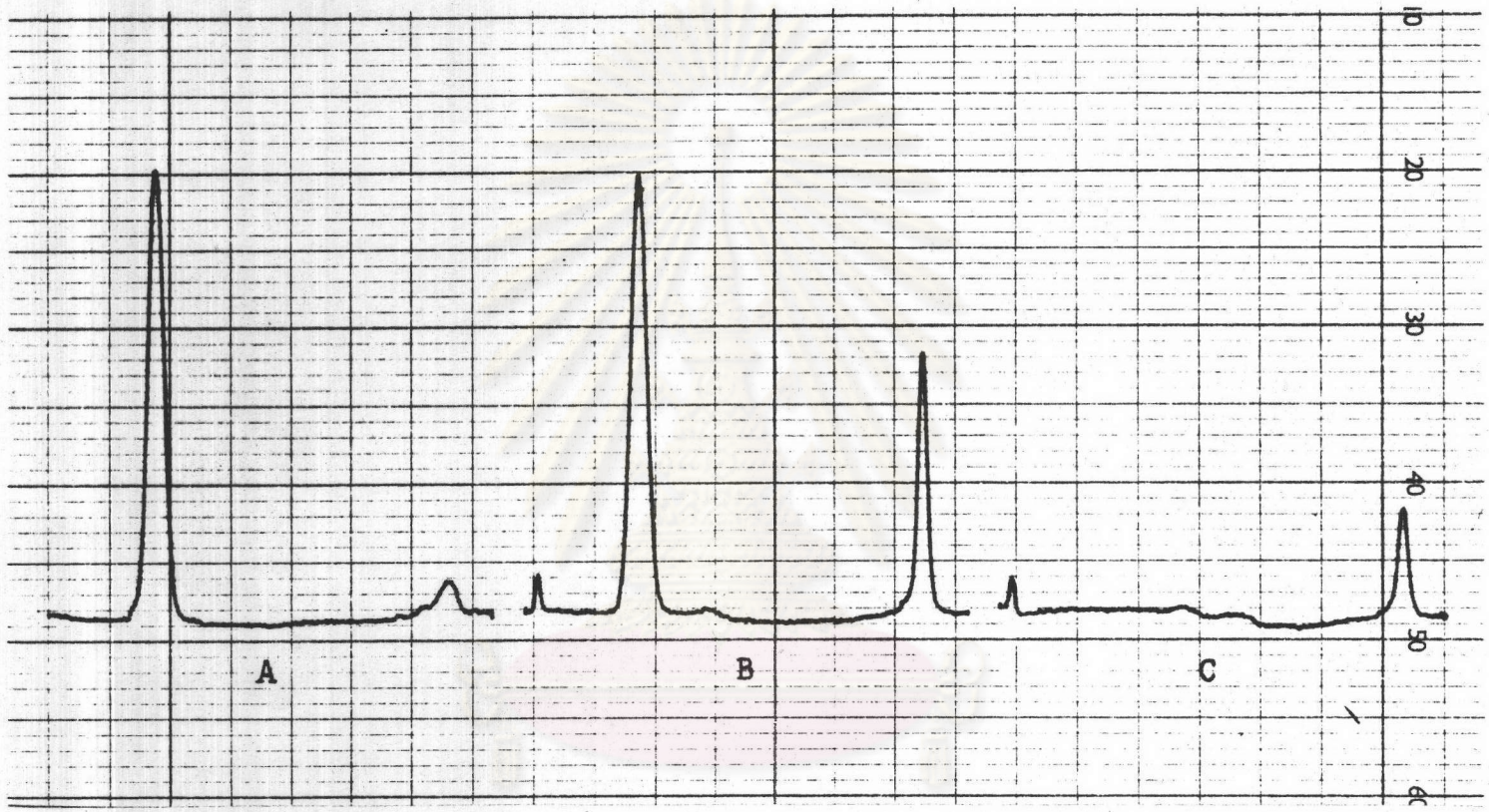

Figure 10. Chromatogram Fluorescence Intensity of

A. Methyltestosterone

B. Mixture of methyltestosterone and cottonseed oil

C. Cottonseed oil

BIOGRAPHY

Miss Yaovalak Wattanapicis was born on the 1st November, 1952, graduated with a B.Sc. in Pharmacy (Honour) from Chulalongkorn University in 1975 and is now working in Drug Analysis Division, Department of Medical Sciences, Ministry of Public Health.