REFERENCES

1. Weigele, M., DeBernardo, S.L., Tengi, J.P. and Leimgruber, W., "A Novel Reagent for the Fluorometric Assay of Primary Amines." J.Amer.Chem.Soc. 94(16), (1972) : 5927-5928.

2. Weigele, M., Tengi, J.P., DeBernardo, S., Czajkowski, R. and Leimgruber, W., "Fluorometric Reagents for Primary Amines." J.Org.Chem. 41(2), (1976) : 388-389.

3. Felix, A.M., Toome, V., DeBernardo, S. and Weigele, M., "Colorimetric Amino Acid Analysis Using Fluorescamine." Arch.Biochem.Biophys. 168(2), (1975) : 601-608.

4. Felix, A.M. and Terkelsen, G., "Determination of Hydroxyproline in Fluorometric Amino Acid Analysis with Fluorescamine." Anal.Biochem. 56(2), (1973) : 610-615.

5. Felix, A.M., Westley, J.W. and Meienhofer, J., "Fluorometric - Colorimetric Amino Acid Analysis of Actinomycins Using Fluorescamine." Anal.Biochem. 73(1), (1976) : 70-77.

6. Stein, S., Böhlen, P., Stone, J., Dairman, W. and Udenfriend, S., "Amino Acid Analysis with Fluorescamine at the Picomole Level." Arch.Biochem.Biophys. 155(1), (1973) : 203-212.

7. Felix, A.M., and Terkelsen, G., "Total Fluorometric Amino Acid Analysis Using Fluorescamine." Arch.Biochem.Biophys. 157, (1973) : 177-182.

8. Felix, A.M. and Terkelsen, G., "Fluorometric Analysis of N Methylamino Acids Using Fluorescamine." Anal.Biochem. 60, (1974) : 78-87.

9. Udenfriend, S., Stein, S., Böhlen, P., Dairman, W., Leimgruber, W. and Weigele, M., "Fluorescamine : A Reagent for Assay of Amino Acids, Peptides, Proteins, and Primary Amines in the Picomole Range." Science 178, (1972) : 871-872.

10. Perrett, D., Webb, J.P.W., Silk, D.B.A. and Clark, M., "The Assay of Dipeptides using Fluorescamine and its Application to Determining Dipeptidase Activity." Anal.Biochem. 68(1), (1975) : 161-166.

11. Böhlen, P., Stein, S., Dairman, W. and Udenfriend, S., "Fluorometric Assay of Proteins in the Nanogram Range." Arch.Biochem. 155(1), (1973) : 213-220.

12. Toome, V. and Wegrzynski, B., "Chiroptical Properties of Fluorescamine Condensation Compounds with Amino Acid Esters in situ. Pyrrolinone Chirality Rule." Biochem.Biophys. Res.Commun. 85(4), (1978) : 1496-1502.

13. Toome, V., Wegrzynski, B. and Dell, J., "Chiroptical Properties of Fluorescamine Condensation Compounds with Dipeptides in situ." Biochem.Biophys.Res.Commun. 74(2), (1977) : 825-830.

14. Kovacs, K.L., "Chiroptical Studies of Fluorescamine Labelled Amino Acids." Biochem.Biophys.Res.Commun. 86(4), (1979) : 995-1001.

15. Stein, S., Böhlen, P. and Udenfriend, S., "Studies on the Kinetics of Reaciton and Hydrolysis of Fluorescamine." Arch.Biochem.Biophys. 163(1), (1974) : 400-403.

16. Cheng, L. K., Levitt, M. and Fung H.L., "Fluorescence Reactions of Fluorescamine with Levodopa and Its Derivatives : Fluorescence Assay of 3-Methoxy-4-hydroxyphenylalanine in Levodopa Dosage Forms." J.Pharm.Sci. 64(5), (1975) : 839-841.

17. Silva, J.A.F. and Strojny, N., "Spectrofluorometric Determination of Pharmaceuticals Containing Aromatic or Aliphatic Primary Amino Groups as Their Fluorescamine (Fluram) Derivatives." Anal.Chem. 47(4), (1975) : 741-718.

18. Sterling, J.M. and Haney, W.G., "Spectrophotofluorometric Analysis of Procainamide and Sulfadiazine in Presence of Primary Aliphatic Amines Based on Reaction with Fluorescamine." J.Pharm.Sci. 63(9), (1974) : 1448-1450.

19. Imai, K., "Fluorimetric Assay of Dopamine, Norepinephrine, and Their-O-Methyl Metabolites by Using Fluorescamine." J.Chromatography. 105(1), (1975) : 135-140.

20. Caddy, B. and Stead, A.H., "Some Fluorescent Derivatives of the Drug Phenelzine." Analyst. 103, (1978) : 937-949.

21. Fabregas, J. L. and Beneyto, J.E., "Direct Spectrofluorimetric Determination of the Free Amino Group of Cephalexin in its Lysine Salt." Analyst. 105, (1980) : 813-816.

22. Fourche, L., Jensen, H. and Neuzil, E., "Fluorescence Reactions of Aminophosphonic Acids." Anal.Chem. 48(1), (1976) : 155-159.

23. Eckstein, Y. and Dreyfuss, P., "Determination of Primary Amine Endogroups on Polymers with Fluorescamine in Nonaqueous Solvents." Anal.Chem. 52(3), (1980) : 531-541.

24. Toom, V., Wegrzynski, B. and Dell, J., "Chiroptical Properties of Fluorescamine Condensation Compounds with Secondary Amino Acids in situ." Biochem.Biophys.Res.Commun. 71(2), (1976) : 598-602.

25. Narasimhachari, N., "Mass Spectra of the Reaction Products of Some Biologically Important Primary Amines and Fluorescamine." Biochem.Biophys.Res.Commun. 55(1), (1973) : 231-238.

26. Froehlich, P.M. and Murphy, L.D., "Enhancement of the Fluorescence Intensity of Derivatives of Amino Acids in Mixed Solvent System." Anal.Chem. 49(11), (1977) : 1606-1608.

27. Nakamura, H. and Pisano, J.J., "Reaction of Tryptophan, Tryptamines, and Some Related Indoles with Fluorescamine : Unique Fluorescence in Strong Acid." Arch.Biochem. Biophys. 172(1), (1976) : 98-101.

28. Felix, A.M. and Jimenez, M.H., "Rapid Fluorometric Detection for Completeness in Solid Phase Coupling Reactions." Anal. Biochem. 52(2), (1973) : 377-381.

29. Ranieri, R.L. and McLaughlin, J.L., "Cactus Alkaloids XXVII. Use of Fluorescamine as a Thin-Layer Chromatographic Visualization Reagent for Alkaloids." J.Chromatography, 111, (1975) : 234-237.

30. Imai, K., Böhlen, P., Stein, S. and Udenfriend, S., "Detection of Fluorescamine-Labeled Amino Acids, Peptides, and Other Primary Amines on Thin-Layer Chromatograms." Arch. Biochem.Biophys. 161(1), (1974) : 161-163.

31. Felix, A.M. and Jimenez, M.H., "Usage of Fluorescamine as a Spray Reagent for Thin-Layer Chromatography." J.Chromatography. 89, (1974) : 361-364.

32. Doetsch, P.W., Cassady, J.M. and McLaughlin, J.L., "Cactus Alkaloids XL. Identification of Mescaline and Other β-Phenethylamines in *Pereskia*, *Pereskiopsis* and *Islaya* by Use of Fluorescamine Conjugates." J.Chromatography. 189, (1980) : 79-85.

33. Sherma, J. and Marzoni, G., "Detection and Quantitation of Anilines by TLC Using Fluorescamine Reagent." Int.Lab. Nov. - Dec., (1974) : 41-42, 46, 48-50, 52.

34. Nakamura, H., Sensitive and Specific Detection of Tryptophan, Tryptamine and N-Terminal Tryptophan Peptides on Thin - Layer Plates Using a Unique Fluorogenic Reaction with Fluorescamine." J.Chromatography. 152, (1978) : 153-165.

35. Weeks, R.W., Yasuda, S.K. and Dean, B.J., "Fluorescent Detection of Hydrazines via Fluorescamine and Isomeric Phthalaldehydes." Anal.Chem. 48(1), (1976) : 159-161.

36. Nakamura, H., "Specific Detection of Primary Catecholamines and Their 3-O-Methyl Derivatives on Thin-Layer Plates Using a Fluorigenic Reaction with Fluorescamine." J.Chromatography. 154, (1978) : 51-59.

37. Weigele, M., DeBernardo, S. and Leimgruber, W., "Fluorometric Assay of Secondary Amino Acids." Biochem.Biophys.Res. Commun. 50(2), (1973) : 352-356.

38. Nakamura, H. and Tamura, Z., "Fluorometric Determination of Secondary Amines Based on Their Reaction with Fluorescamine." Anal.Chem. 52, (1980) : 2087-2092.

39. DeBernardo, S., Weigele, M., Toome, V., Manhart, K. and Leimgruber, W., "Studies on the Reaction of Fluorescamine with Primary Amines." Arch.Biochem.Biophys. 163(1), (1974) : 390-399.

40. Chen, R.F., Smith, P.D. and Maly, M., "The Fluorescence of Fluorescamine-Amino Acids." Arch.Biochem.Biophys. 189(2), (1978) : 241-250.

41. Kice, J.L. and Marwell, E.N. Modern Principles of Organic Chemistry. pp. 256, 260, 261, Amerind Publishing Co. Rvt. Ltd., New York, 1972.

42. March, J. Advanced Organic Chemistry Reactions, Mechanism and Structure. 2d ed. pp. 265-266, 270, 377, 382-384, 386, 451, 520, 525, 580, 582, 584, 587, 588, 589, 672, 678, 680-682, 704-706, 738, 755, 801, 802, 817, 818, 820, 821, 1085, 1086, 1106, 1107, Mc Graw-Hill Book Company, New York, 1977.

43. Prescott, F. and Ridge, D. Organic Chemistry. A Textbook for Science and Medical Students. 2d ed. pp. 209, 210, 463, University Tutorial Press Ltd., London, 1950.

44. Richter, G.H. Textbook of Organic Chemistry. 3d ed. pp. 245-247, 249-250, John Wiley & Sons, Inc., New York, 1952.

45. Brewster, R.Q. and McEwen, W.E. Organic Chemistry. 3d ed. pp. 284, 289, Prentice-Hall, Inc., New York, 1961.

46. Turner, E.E. and Harris, M.M. Organic Chemistry. Third Impression with Corrections and Additions. p. 143, Spottiswoode, Ballantyne and Co., London, 1960.

47. Fieser, L.F. and Fieser, M. Advanced Organic Chemistry. Maruzen Asian Edition, pp. 497, 516, 717, Reinhold Publishing Corporation Maruzen Company, Limited, Tokyo, 1961.

48. Siggia, S. Quantitative Organic Analysis via Functional Groups pp. 65-71, John Wiley & Sons, Inc., New York, 1949.

49. Stone, K.G. Determination of Organic Compounds. pp. 165-180, McGraw-Hill Book Company, Inc., New York, 1956.

50. Connors, K.A. A Textbook of Pharmaceutical Analysis. 2d ed. pp. 508-515, A Willey-Interscience Publication, New York, 1967.

51. Official Methods of Analysis of the Association of Official Analytical Chemists. 13 th ed. pp. 636, 660-663, Association of Official Analytical Chemists, Washington, DC, 1980.

52. British Pharmacopoeia 1980. pp. 374-375, 379, 557, 650, 659, 764-765, 815, London Her Majesty's Stationery Office, Cambridge, 1980.

53. Garratt, D.C. The Quantitative Analysis of Drugs. 3d ed. pp. 19-21, 233-239, 536-537, Chapman & Hall Toppan, London, 1964.

54. Specifications for the Quality Control of Pharmaceutical Preparations. second edition of the international Pharmacopoeia World Health Organization, pp. 186-189, World Health Organization, Geneva, 1967.

55. The United States Pharmacopoeia. 20 th revision, The National Formulary. 15 th ed. pp. 276, 683-684, United States Pharmacopeial Convention, Inc., Rockville, Md., 1980.

56. The National Formulary. 14 th ed. pp. 626-627, 894, American Pharmaceutical Association, Washington, DC., 1975.

57. The United States Pharmacopoeia. 19 th revision, pp. 170-172, 386-388, United States Pharmacopeial Convention, Inc., Rockville, Md., 1975.

58. Gearien, J.E. and Grabowski, B.F. Method of Drug Analysis. p. 56, Lea & Febiger, Philadelphia, 1969.

59. Fritz, J.S. and Hammond, G.S. Quantitative Organic Analysis. p. 43, John Wiley & Sons, Inc., New York, 1957.

60. Higuchi, T. and Bodin, J.I. Alkaloids and Other Basic Nitrogenous Compounds. in Pharmaceutical Analysis. (Higuchi, T. and Brochmann-Hanssen, E. eds.) pp. 375-378, 410-411, 413-418, 448, 450, 453, 460-462, Hanssen Interscience Publishers, New York, 1961.

61. Harvey, S.C. Adrenergic Blocking Drugs. in Remington's Pharmaceutical Sciences. (Osol, A., Chase, G.D., Gennaro, A.R., Gibson, M.R., Granberge, C.B., Harvey, S.C., King, R.E., Martin, A.N., Swinyard, E.A. and Zink, G.L. eds.) 16 th ed. p. 846, Mack Publishing Company, Pennsylvania, 1980.

62. Harvey, S.C. Sympathomimetic Drugs in Remington's Pharmaceutical Sciences. (Osol, A., Hoover, J.E., Anderson, J.T., Bendush, C.L., Chase, G.D., Gennaro, A.R., Gibson, M.R., Granberg, C.B., Harvey, S.C., King, R.E., Martin, A.N.

and Swinyard, E.A. eds.) 15 th ed. pp. 811-813, 819-820, Mack Publishing Company, Pennsylvania, 1975.

63. Windholz, M., Budavari, S., Stroumtsos, L.Y. and Fertig, M.N. (eds) The Merck Index An Encyclopedia of Chemicals and Drugs. 9 th ed. pp. 802-803, Marck & Co., Inc., New Jersy, 1976.

64. Swinyard, E.A. Parasiticides. in Remington's Pharmaceutical Sciences. (Osol, A., Hoover, J.E., Anderson, J.T., Bendush, C.L., Chase, G.D., Gennaro, A.R., Gibson, M.R., Granberg, C.B., Harvey, S.C., King, R.E., Martin, A.N. and Swinyard, E.A. eds.) 15 th ed. p. 1175, Mack Publishing Company, Pennsylvania, 1975.

APPENDIX

Table 1 Maximum Absorption of Secondary Amine Drug-Fluorescamine Derivatives

Drugs	Maximum Absorption, nm
Ephedrine HCl	317
Pseudoephedrine HCl	317
Phenylephrine HCl	317
Epinephrine bitartrate	317
Metoprolol tartrate	317
Propranolol HCl	319
Piperazine citrate	325

Table 2 Effect of pH on the Formation of Ephedrine-Fluorescamine Derivative

pH	Absorbance[a] at 317 nm	% CV
2	0.000 ± 0.000	0.00
3	0.000 ± 0.000	0.00
4	0.000 ± 0.000	0.00
5	0.000 ± 0.000	0.00
6	0.000 ± 0.000	0.00
7	0.381 ± 0.010	2.73
8	0.932 ± 0.015	1.50
9	1.003 ± 0.015	1.50
10	0.945 ± 0.011	1.11
11	0.538 ± 0.032	6.02
12	0.052 ± 0.010	18.98

[a]Mean value ± S.D. of four experiments

Table 3 Effect of pH on the Formation of Pseudoephedrine-Fluorescamine Derivative

pH	Absorbance[a] at 317 nm	% CV
2	0.000 ± 0.000	0.00
3	0.000 ± 0.000	0.00
4	0.000 ± 0.000	0.00
5	0.000 ± 0.000	0.00
6	0.000 ± 0.000	0.00
7	0.323 ± 0.009	2.79
8	0.968 ± 0.010	1.00
9	1.030 ± 0.010	0.94
10	1.004 ± 0.008	0.76
11	0.598 ± 0.010	1.74
12	0.028 ± 0.004	13.54

[a]Mean value ± S.D. of four experiments

Table 4 Effect of pH on the Formation of Phenylephrine-Fluorescamine Derivative

pH	Absorbance[a] at 317 nm	% CV
2	0.000 ± 0.000	0.00
3	0.000 ± 0.000	0.00
4	0.000 ± 0.000	0.00
5	0.000 ± 0.000	0.00
6	0.000 ± 0.000	0.00
7	0.727 ± 0.008	1.15
8	0.890 ± 0.006	0.70
9	0.907 ± 0.006	0.71
10	0.912 ± 0.009	0.99
11	0.817 ± 0.005	0.62
12	0.092 ± 0.003	3.55

[a]Mean value ± S.D. of four experiments

Table 5 Effect of pH on the Formation of Epinephrine-Fluorescamine Derivative

pH	Absorbance[a] at 317 nm	% CV
2	0.000 ± 0.000	0.00
3	0.000 ± 0.000	0.00
4	0.000 ± 0.000	0.00
5	0.000 ± 0.000	0.00
6	0.000 ± 0.000	0.00
7	0.640 ± 0.007	1.09
8	0.938 ± 0.007	0.72
9	0.974 ± 0.010	1.00
10	0.850 ± 0.002	0.27
11	0.700 ± 0.008	1.20
12	0.617 ± 0.008	1.25

[a]Mean value ± S.D. of four experiments

Table 6 Effect of pH on the Formation of Metoprolol-Fluorescamine Derivative

pH	Absorbance[a] at 317 nm	% CV
2	0.000 ± 0.000	0.00
3	0.000 ± 0.000	0.00
4	0.000 ± 0.000	0.00
5	0.000 ± 0.000	0.00
6	0.000 ± 0.000	0.00
7	0.068 ± 0.008	11.94
8	0.388 ± 0.010	2.51
9	0.625 ± 0.005	0.76
10	0.854 ± 0.006	0.74
11	0.170 ± 0.003	1.55
12	0.000 ± 0.000	0.00

[a]Mean value ± S.D. of four experiments

Table 7 Effect of pH on the Formation of Propranolol-Fluorescamine Derivative

pH	Absorbance[a] at 319 nm	% CV
2	0.124 ± 0.003	2.51
3	0.123 ± 0.002	1.80
4	0.114 ± 0.006	4.83
5	0.095 ± 0.005	5.11
6	0.148 ± 0.004	2.37
7	0.170 ± 0.004	2.59
8	0.381 ± 0.009	2.24
9	0.687 ± 0.005	0.79
10	0.761 ± 0.008	1.04
11	0.422 ± 0.005	1.12
12	0.191 ± 0.006	3.13

[a]Mean value ± S.D. of four experiments

Table 8 Effect of pH on the Formation of Piperazine-Fluorescamine Derivative

pH	Absorbance[a] at 325 nm	% CV
2	0.000 ± 0.000	0.00
3	0.000 ± 0.000	0.00
4	0.000 ± 0.000	0.00
5	0.000 ± 0.000	0.00
6	0.000 ± 0.000	0.00
7	0.503 ± 0.005	0.95
8	0.772 ± 0.009	1.19
9	0.790 ± 0.010	1.24
10	0.842 ± 0.010	1.16
11	0.603 ± 0.012	2.01
12	0.000 ± 0.000	0.00

[a]Mean value ± S.D. of four experiments

Table 9 Effect of Time on Absorbance of Ephedrine-Fluorescamine Derivative

Time (minutes)	Absorbance[a] at 317 nm	% CV
2	1.035 ± 0.004	0.39
5	1.039 ± 0.006	0.56
10	1.039 ± 0.006	0.60
15	1.039 ± 0.006	0.60
20	1.039 ± 0.006	0.60
25	1.037 ± 0.004	0.40
30	1.034 ± 0.004	0.35
35	1.028 ± 0.005	0.52
40	1.028 ± 0.004	0.37
45	1.024 ± 0.006	0.54
50	1.024 ± 0.006	0.55
55	1.024 ± 0.006	0.55
60	1.016 ± 0.006	0.63
90	1.007 ± 0.005	0.52
120	0.996 ± 0.006	0.64
150	0.989 ± 0.008	0.83
180	0.983 ± 0.006	0.61
1,440	0.645 ± 0.005	0.74

[a]Mean value ± S.D. of four experiments

Table 10 Effect of Time on Absorbance of Pseudoephedrine-Fluorescamine Derivative

Time (minutes)	Absorbance[a] at 317 nm	% CV
2	0.984 ± 0.013	1.34
5	0.984 ± 0.013	1.34
10	0.984 ± 0.011	1.09
15	0.984 ± 0.011	1.09
20	0.981 ± 0.010	1.00
25	0.980 ± 0.010	0.97
30	0.976 ± 0.009	0.93
35	0.973 ± 0.009	0.91
40	0.968 ± 0.009	0.93
45	0.963 ± 0.009	0.89
50	0.958 ± 0.008	0.88
55	0.953 ± 0.009	0.92
60	0.948 ± 0.008	0.83
90	0.944 ± 0.012	1.25
120	0.932 ± 0.016	1.67
150	0.920 ± 0.014	1.55
180	0.908 ± 0.017	1.88
1,440	0.481 ± 0.012	2.54

[a]Mean value ± S.D. of four experiments

Table 11 Effect of Time on Absorbance of Phenylephrine-Fluorescamine Derivative

Time (minutes)	Absorbance[a] at 317 nm	% CV
2	0.985 ± 0.006	0.56
5	0.982 ± 0.008	0.80
10	0.980 ± 0.008	0.81
15	0.977 ± 0.007	0.75
20	0.974 ± 0.006	0.61
25	0.969 ± 0.007	0.74
30	0.969 ± 0.006	0.60
35	0.966 ± 0.005	0.55
40	0.960 ± 0.004	0.42
45	0.953 ± 0.005	0.56
50	0.948 ± 0.006	0.59
55	0.944 ± 0.004	0.41
60	0.945 ± 0.006	0.58
90	0.914 ± 0.006	0.63
120	0.899 ± 0.004	0.45
150	0.885 ± 0.005	0.59
180	0.865 ± 0.005	0.59
1,440	0.480 ± 0.008	1.60

[a]Mean value ± S.D. of four experiments

Table 12 Effect of Time on Absorbance of Epinephrine-Fluorescamine Derivative

Time (minutes)	Absorbance[a] at 317 nm	% CV
2	0.967 ± 0.007	0.73
5	0.960 ± 0.008	0.80
10	0.952 ± 0.007	0.78
15	0.943 ± 0.007	0.75
20	0.932 ± 0.008	0.81
25	0.924 ± 0.007	0.79
30	0.917 ± 0.008	0.87
35	0.910 ± 0.008	0.90
40	0.902 ± 0.007	0.79
45	0.897 ± 0.009	0.98
50	0.890 ± 0.009	1.04
55	0.885 ± 0.008	0.89
60	0.879 ± 0.007	0.82
90	0.864 ± 0.006	0.71
120	0.846 ± 0.009	1.02
150	0.829 ± 0.008	0.95
180	0.814 ± 0.006	0.72
1,440	0.480 ± 0.009	1.90

[a]Mean value ± S.D. of four experiments

Table 13 Effect of Time on Absorbance of Metoprolol-Fluorescamine Derivative

Time (minutes)	Absorbance[a] at 317 nm	% CV
2	0.774 ± 0.004	0.53
5	0.758 ± 0.008	1.03
10	0.757 ± 0.009	1.22
15	0.752 ± 0.010	1.30
20	0.749 ± 0.010	1.33
25	0.749 ± 0.009	1.16
30	0.747 ± 0.008	1.08
35	0.742 ± 0.008	1.09
40	0.737 ± 0.008	1.14
45	0.733 ± 0.009	1.23
50	0.732 ± 0.010	1.32
55	0.735 ± 0.010	1.38
60	0.731 ± 0.011	1.38
90	0.711 ± 0.006	0.79
120	0.688 ± 0.010	1.40
150	0.677 ± 0.008	1.19
180	0.647 ± 0.009	1.34
1,440	0.268 ± 0.009	3.17

[a]Mean value ± S.D. of four experiments

Table 14 Effect of Time on Absorbance of Propranolol-Fluorescamine Derivative

Time (minutes)	Absorbance[a] at 319 nm	% CV
2	0.765 ± 0.007	0.87
5	0.758 ± 0.008	1.08
10	0.756 ± 0.009	1.21
15	0.755 ± 0.010	1.30
20	0.754 ± 0.011	1.42
25	0.753 ± 0.011	1.43
30	0.753 ± 0.011	1.47
35	0.752 ± 0.012	1.55
40	0.751 ± 0.011	1.52
45	0.752 ± 0.012	1.53
50	0.752 ± 0.012	1.55
55	0.750 ± 0.012	1.56
60	0.749 ± 0.011	1.51
90	0.745 ± 0.011	1.47
120	0.738 ± 0.011	1.55
150	0.734 ± 0.012	1.62
180	0.731 ± 0.011	1.48
1,440	0.468 ± 0.007	1.41

[a]Mean value ± S.D. of four experiments

Table 15 Effect of Time on Absorbance of Piperazine-Fluorescamine Derivative

Time (minutes)	Absorbance[a] at 325 nm	% CV
2	0.633 ± 0.002	0.39
5	0.622 ± 0.002	0.38
10	0.604 ± 0.004	0.71
15	0.591 ± 0.005	0.81
20	0.578 ± 0.004	0.72
25	0.563 ± 0.006	1.11
30	0.554 ± 0.008	1.41
35	0.544 ± 0.007	1.31
40	0.535 ± 0.007	1.31
45	0.526 ± 0.007	1.41
50	0.515 ± 0.009	1.65
55	0.508 ± 0.008	1.64
60	0.497 ± 0.011	2.21
90	0.468 ± 0.005	1.12
120	0.430 ± 0.007	1.52
150	0.404 ± 0.006	1.46
180	0.382 ± 0.007	1.77
1,440	0.082 ± 0.006	7.87

[a]Mean value ± S.D. of four experiments

Table 16 Effect of Temperature on Absorbance of Secondary Amine Drug-Fluorescamine Derivatives

Temperature / Drugs	30℃		40℃		50℃	
	Absorbance[a]	% CV	Absorbance[a]	% CV	Absorbance[a]	% CV
Ephedrine HCl[b]	1.055±0.010	0.92	1.040±0.008	0.79	0.973±0.006	0.66
Pseudophedrine HCl[b]	1.055±0.005	0.52	0.951±0.006	0.63	0.910±0.013	1.44
Phenylephrine HCl[b]	0.969±0.010	1.02	0.936±0.009	0.91	0.869±0.013	1.48
Epinephrine bitatrate[b]	0.931±0.006	0.60	0.864±0.006	0.72	0.752±0.007	0.99
Metoprolol tartrate[b]	1.054±0.009	0.83	1.015±0.014	1.35	0.972±0.008	0.83
Propranolol HCl[c]	0.732±0.006	0.88	0.702±0.013	1.86	0.680±0.009	1.31
Piperazine citrate[d]	1.040±0.009	0.85	0.985±0.007	0.74	0.817±0.017	2.06

[a]Mean value ± S.D. of four experiments

[b]Absorbance at 317 nm

[c]Absorbance at 319 nm

[d]Absorbance at 325 nm

Table 17 Effect of Fluorescamine Concentration on Absorbance of Ephedrine-Fluorescamine Derivative

conc of Fluorescamine x 10^{-4} M	Mole Ratio Fluorescamine to Ephedrine	Absorbance[a] at 317 nm	% CV
1	1:2	0.164 ± 0.003	1.75
2	1:1	0.298 ± 0.009	3.06
4	2:1	0.468 ± 0.011	2.33
6	3:1	0.491 ± 0.007	1.39
8	4:1	0.525 ± 0.007	1.40
10	5:1	0.520 ± 0.008	1.58
12	6:1	0.511 ± 0.005	0.93
14	7:1	0.520 ± 0.010	1.86
16	8:1	0.521 ± 0.008	1.58
18	9:1	0.523 ± 0.004	0.82
20	10:1	0.502 ± 0.007	1.43

[a]Mean value ± S.D. of four experiments

Table 18 Effect of Fluorescamine Concentration on Absorbance of Pseudoephedrine-Fluorescamine Derivative

conc of Fluorescamine x 10^{-4} M	Mole Ratio Fluorescamine to Pseudoephedrine	Absorbance[a] at 317 nm	% CV
1	1:2	0.135 ± 0.001	0.61
2	1:1	0.257 ± 0.003	1.02
4	2:1	0.405 ± 0.005	1.35
6	3:1	0.476 ± 0.008	1.68
8	4:1	0.482 ± 0.012	2.59
10	5:1	0.489 ± 0.003	0.61
12	6:1	0.483 ± 0.007	1.37
14	7:1	0.478 ± 0.008	1.73
16	8:1	0.489 ± 0.007	1.53
18	9:1	0.484 ± 0.005	0.96
20	10:1	0.473 ± 0.008	1.66

[a]Mean value ± S.D. of four experiments

Table 19 Effect of Fluorescamine Concentration on Absorbance of Phenylephrine-Fluorescamine Derivative

conc of Fluorescamine x 10^{-4} M	Mole Ratio Fluorescamine to Phenylephrine	Absorbance[a] at 317 nm	% CV
1	1:2	0.221 ± 0.002	0.68
2	1:1	0.415 ± 0.003	0.80
4	2:1	0.481 ± 0.007	1.54
6	3:1	0.481 ± 0.008	1.75
8	4:1	0.477 ± 0.010	2.09
10	5:1	0.478 ± 0.008	1.72
12	6:1	0.475 ± 0.008	1.62
14	7:1	0.475 ± 0.012	2.44
16	8:1	0.479 ± 0.010	2.17
18	9:1	0.478 ± 0.009	1.91
20	10:1	0.463 ± 0.005	1.16

[a]Mean value ± S.D. of four experiments

Table 20 Effect of Fluorescamine Concentration on Absorbance of Epinephrine-Fluorescamine Derivative

conc of Fluorescamine x 10^{-4} M	Mole Ratio Fluorescamine to Epinephrine	Absorbance[a] at 317 nm	% CV
1	1:2	0.250 ± 0.008	3.21
2	1:1	0.415 ± 0.006	1.54
4	2:1	0.494 ± 0.005	1.02
6	3:1	0.500 ± 0.008	1.64
8	4:1	0.502 ± 0.008	1.55
10	5:1	0.495 ± 0.007	1.43
12	6:1	0.499 ± 0.010	2.09
14	7:1	0.500 ± 0.014	2.71
16	8:1	0.495 ± 0.007	1.35
18	9:1	0.497 ± 0.008	1.54
20	10:1	0.498 ± 0.016	3.19

[a]Mean value ± S.D. of four experiments

Table 21 Effect of Fluorescamine Concentration on Absorbance of Metoprolol-Fluorescamine Derivative

conc of Fluorescamine x 10^{-4} M	Mole Ratio Fluorescamine to Metoprolol	Absorbance[a] at 317 nm	% CV
2	1:2.4	0.094 ± 0.007	7.43
4	1:1.2	0.166 ± 0.005	3.05
8	1.6:1	0.274 ± 0.004	1.47
12	2.5:1	0.387 ± 0.008	1.97
16	3.3:1	0.471 ± 0.009	1.85
20	4.1:1	0.490 ± 0.007	1.53
24	4.9:1	0.531 ± 0.011	2.04
28	5.7:1	0.542 ± 0.006	1.15

[a]Mean value ± S.D. of four experiments

Table 22 Effect of Fluorescamine Concentration on Absorbance of Propranolol-Fluorescamine Derivative

conc of Fluorescamine x 10^{-4} M	Mole Ratio Fluorescamine to Propranolol	Absorbance[a] at 319 nm	% CV
1	1:2	0.109 ± 0.003	2.70
2	1:1	0.142 ± 0.002	1.20
4	2:1	0.186 ± 0.005	2.58
6	3:1	0.238 ± 0.002	0.99
8	4:1	0.269 ± 0.007	2.60
10	5:1	0.299 ± 0.002	0.38
12	6:1	0.313 ± 0.005	1.52
14	7:1	0.315 ± 0.005	1.67
16	8:1	0.351 ± 0.008	2.14
18	9:1	0.364 ± 0.004	0.96
20	10:1	0.359 ± 0.006	1.60

[a]Mean value ± S.D. of four experiments

Table 23 Effect of Fluorescamine Concentration on Absorbance of Piperazine-Fluorescamine Derivative

conc of Fluorescamine x 10^{-4} M	Mole Ratio Fluorescamine to Piperazine	Absorbance[a] at 325 nm	% CV
0.5	1:2	0.125 ± 0.008	6.07
1	1:1	0.199 ± 0.006	2.88
2	2:1	0.395 ± 0.005	1.33
3	3:1	0.501 ± 0.003	0.60
4	4:1	0.520 ± 0.017	3.18
5	5:1	0.507 ± 0.012	2.40
6	6:1	0.512 ± 0.008	1.60
7	7:1	0.515 ± 0.013	2.53
8	8:1	0.512 ± 0.009	1.81
9	9:1	0.510 ± 0.004	0.86
10	10:1	0.511 ± 0.012	2.33
15	15:1	0.505 ± 0.009	1.81
20	20:1	0.504 ± 0.004	0.88

[a]Mean value ± S.D. of four experiments

Table 24 Absorbance-Concentration Relationship of Ephedrine HCl

conc Ephedrine HCl mcg/ml	Absorbance[a] at 317 nm	% CV
4.04	0.243 ± 0.007	2.77
8.08	0.491 ± 0.007	1.46
12.12	0.760 ± 0.006	0.75
16.16	1.002 ± 0.006	0.62
20.20	1.257 ± 0.004	0.35
24.24	1.466 ± 0.006	0.39
28.28	1.711 ± 0.008	0.44
32.32	1.891 ± 0.003	0.13

[a]Mean value ± S.D. of four experiments

Table 25 Absorbance-Concentration Relationship of Pseudoephedrine HCl

conc Pseudoephedrine HCl mcg/ml	Absorbance[a] at 317 nm	% CV
4.04	0.232 ± 0.008	3.27
8.08	0.505 ± 0.004	0.75
12.12	0.771 ± 0.004	0.53
16.16	1.037 ± 0.008	0.75
20.20	1.316 ± 0.015	1.16
24.24	1.559 ± 0.007	0.43
28.28	1.774 ± 0.005	0.27
32.32	1.972 ± 0.009	0.44

[a]Mean value ± S.D. of four experiments

Table 26 Absorbance-Concentration Relationship of Phenylephrine HCl

conc Phenylephrine HCl mcg/ml	Absorbance[a] at 317 nm	% CV
4.08	0.217 ± 0.001	0.44
8.16	0.464 ± 0.008	1.69
12.24	0.710 ± 0.007	0.95
16.32	0.954 ± 0.003	0.28
20.40	1.202 ± 0.006	0.50
24.48	1.438 ± 0.007	0.45
28.56	1.657 ± 0.004	0.27
32.64	1.864 ± 0.006	0.31

[a]Mean value ± S.D. of four experiments

Table 27 Absorbance-Concentration Relationship of Epinephrine bitartrate

conc Epinephrine bitartrate mcg/ml	Absorbance[a] at 317 nm	% CV
6.66	0.251 ± 0.012	4.84
13.32	0.494 ± 0.006	1.24
19.98	0.739 ± 0.006	0.84
26.64	0.969 ± 0.011	1.14
33.30	1.200 ± 0.014	1.18
39.96	1.446 ± 0.018	1.27
46.62	1.693 ± 0.008	0.49
53.28	1.911 ± 0.013	0.69

[a]Mean value ± S.D. of four experiments

Table 28 Absorbance-Concentration Relationship of Metoprolol tartrate

conc Metoprolol tartrate mcg/ml	Absorbance[a] at 317 nm	% CV
8.34	0.141 ± 0.006	3.90
16.68	0.276 ± 0.004	1.45
25.02	0.408 ± 0.002	0.42
33.36	0.548 ± 0.007	1.33
41.70	0.673 ± 0.007	1.07
50.04	0.788 ± 0.006	0.74
58.38	0.859 ± 0.010	1.16
66.72	0.945 ± 0.005	0.51

[a]Mean value ± S.D. of four experiments

Table 29 Absorbance-Concentration Relationship of Propranolol HCl

conc Propranolol HCl mcg/ml	Absorbance[a] at 319 nm	% CV
5.92	0.230 ± 0.017	7.24
11.84	0.422 ± 0.013	2.99
17.76	0.622 ± 0.010	1.65
23.68	0.816 ± 0.011	1.31
29.60	1.014 ± 0.006	0.61
35.52	1.163 ± 0.009	0.80
41.44	1.329 ± 0.016	1.17
47.36	1.483 ± 0.019	1.27

[a]Mean value ± S.D. of four experiments

Table 30 Absorbance-Concentration Relationship of Piperazine citrate

conc Piperazine citrate mcg/ml	Absorbance[a] at 325 nm	% CV
2.14	0.243 ± 0.009	3.56
4.28	0.504 ± 0.007	1.36
6.42	0.759 ± 0.008	1.11
8.56	1.040 ± 0.015	1.41
10.70	1.319 ± 0.004	0.34
12.84	1.590 ± 0.007	0.42
14.98	1.861 ± 0.005	0.26

[a]Mean value ± S.D. of four experiments

Table 31 Linear Concentration Range of Secondary Amine Drugs Using Fluorescamine Method

Drugs	Linear Concentration Range, mcg per ml	slope, ml per mcg	% CV range
Ephedrine HCl	4.04 - 20.20	0.0628	0.13 - 2.77
Pseudoephedrine HCl	4.04 - 20.20	0.0668	0.27 - 3.27
Phenylephrine HCl	4.08 - 24.48	0.0602	0.27 - 1.69
Epinephrine bitartrate	6.66 - 46.62	0.0358	0.49 - 4.84
Metoprolol tartrate	8.34 - 41.70	0.0160	0.42 - 3.90
Propranolol HCl	5.92 - 29.60	0.0331	0.61 - 7.24
Piperazine citrate	2.14 - 14.98	0.1266	0.26 - 3.56

Table 32 Percent Labelled Amount of Propranolol HCl in Propranolol HCl Tablets Using Fluorescamine Method and USP Method

sample	Percent Labelled Amount of Propranolol HCl	
	Fluorescamine Method	USP[a] Method
1	98.74	99.38
2	100.05	99.50
3	100.18	99.62
4	99.27	99.87
5	100.18	99.01
$\overline{X}$	99.68	99.50
SD	0.65	0.21
% CV	0.65	0.21

[a]USP XX

Table 33 Percent Recovery of Propranolol HCl in Propranolol HCl Tablets Using Fluorescamine Method and USP Method (Propranolol HCl added 5 mg)

Sample	Propranolol HCl Added, mg	Fluorescamine Method		USP[a] Method	
		Propranolol HCl found, mg	% Recovery	Propranolol HCl found, mg	% Recovery
1	5.00	4.90	98.08	4.90	97.98
2	5.00	5.07	101.40	4.95	98.98
3	5.00	4.99	99.74	4.95	98.98
4	5.00	5.01	100.29	4.97	99.48
5	5.00	5.07	101.40	5.00	99.98
$\overline{X}$			100.18		99.08
SD			1.38		0.74
% CV			1.37		0.75

[a]USP XX

Table 33 (continued) Percent Recovery of Propranolol HCl in Propranolol HCl Tablets by Fluorescamine Method and USP Method (Propranolol HCl added 10 mg)

Sample	Propranolol HCl Added, mg	Fluorescamine Method		USP[a] Method	
		Propranolol HCl found, mg	% Recovery	Propranolol HCl found, mg	% Recovery
1	10.00	10.24	102.43	10.11	101.10
2	10.00	10.05	100.49	10.09	100.86
3	10.00	10.19	101.88	9.84	98.38
4	10.00	10.02	100.21	10.19	101.85
5	10.00	9.97	99.66	9.94	99.37
$\bar{X}$			100.93		100.31
SD			1.17		1.41
% CV			1.16		1.40

[a]USP XX

Table 33 (continued) Percent Recovery of Propranolol HCl in Propranolol HCl Tablets by Fluorescamine Method and USP Method (Propranolol HCl added 15 mg)

Sample	Propranolol HCl Added, mg	Fluorescamine Method		USP[a] Method	
		Propranolol HCl found, mg	% Recovery	Propranolol HCl found, mg	% Recovery
1	15.00	14.88	99.17	15.18	101.18
2	15.00	15.04	100.27	15.18	101.18
3	15.00	15.15	101.01	15.23	101.51
4	15.00	15.13	100.83	15.13	100.85
5	15.00	14.90	99.35	15.05	100.35
$\bar{X}$			100.13		101.01
SD			0.84		0.44
% CV			0.84		0.43

[a]USP XX

Table 34 Analysis of Propranolol HCl Injection (1 mg per ml) Using Fluorescamine Method and USP Method

Formulation	Label Content, mg	Fluorescamine Method		USP[a] Method	
		Amount Found, mg	% Labelled Amount	Amount Found, mg	% Labelled Amount
Propranolol HCl Injection	1 mg/ml	0.978	97.79	0.982	98.18
		0.986	98.60	0.987	98.68
		0.983	98.30	0.976	97.62
		0.981	98.07	0.997	99.67
		0.989	98.86	0.981	98.06
$\bar{X}$			98.32		98.44
% CV			0.42		0.78

[a]USP XX

Table 35 Analysis of Propranolol HCl Tablets (10 mg per tablet) Using Fluorescamine Method and USP Method

Formulation	Label Content, mg	Fluorescamine Method		USP[a] Method	
		Amount Found, mg	% Labelled Amount	Amount Found, mg	% Labelled Amount
Propranolol HCl Tablet	10 mg/ tablet	9.71	97.10	9.76	97.63
		9.74	97.40	9.76	97.63
		9.81	98.13	9.71	97.14
		9.76	97.64	9.73	97.26
		9.76	97.62	9.79	97.88
$\bar{X}$			97.58		97.51
% CV			0.38		0.30

[a]USP XX

Table 36 Analysis of Propranolol HCl Tablets (40 mg per tablet) Using Fluorescamine Method and USP Method

Formulation	Label Content, mg	Fluorescamine Method		USP[a] Method	
		Amount Found, mg	% Labelled Amount	Amount Found mg	% Labelled Amount
Propranolol HCl Tablet	40 mg/ tablet	40.12	100.29	39.60	99.01
		39.33	98.32	39.75	99.38
		39.26	98.15	39.70	99.25
		39.42	98.56	39.60	99.01
		39.06	97.64	39.85	99.62
$\bar{X}$			98.59		99.25
% CV			1.01		0.26

[a]USP XX

Table 37 Comparative Analysis of Preparation Containing Propranolol HCl

Formulation	Fluorescamine Method		USP[a] Method	
	% Labelled Amount	% CV	% Labelled Amount	% CV
Propranolol HCl Injection 1 mg/ml	98.32	0.42	98.44	0.78
Propranolol HCl Tablet 10 mg/ tablet	97.58	0.38	97.51	0.30
Propranolol HCl Tablet 40 mg/ tablet	98.59	1.01	99.25	0.26

[a]USP XX

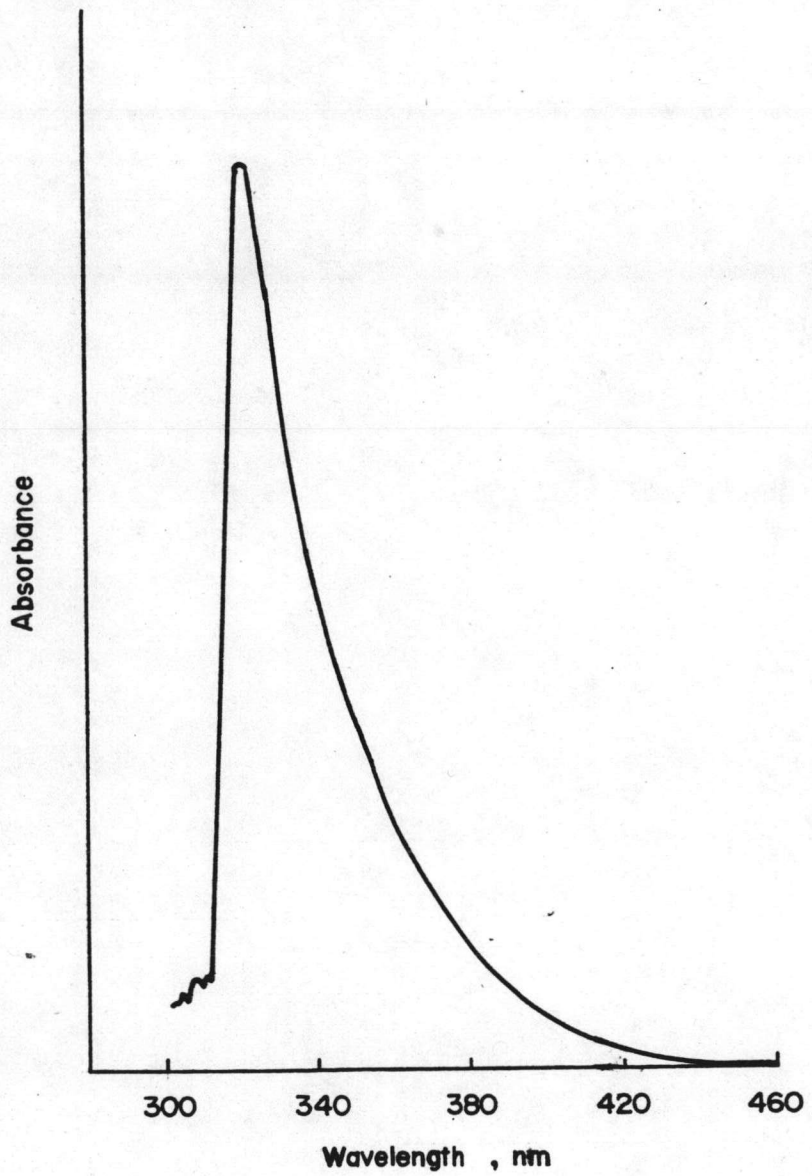

Figure 1. Absorption spectrum of ephedrine - fluorescamine derivative.

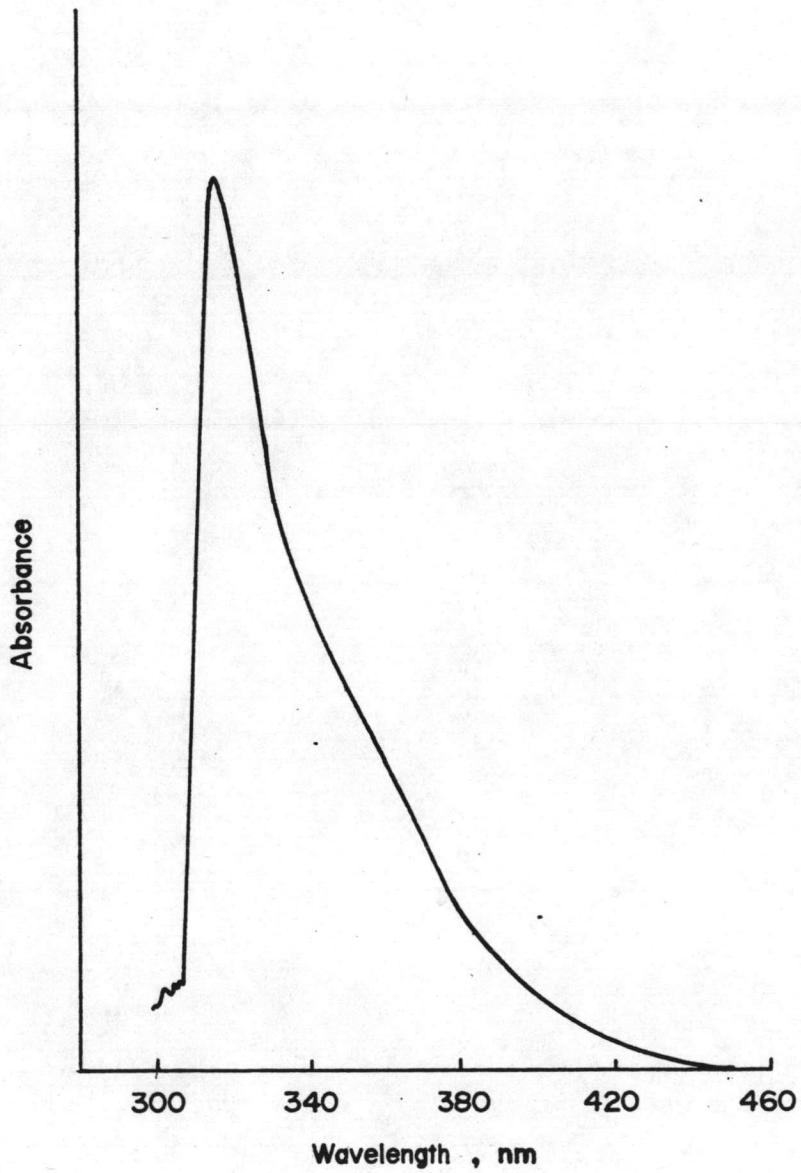

Figure 2. Absorption spectrum of pseudoephedrine - fluorescamine derivative.

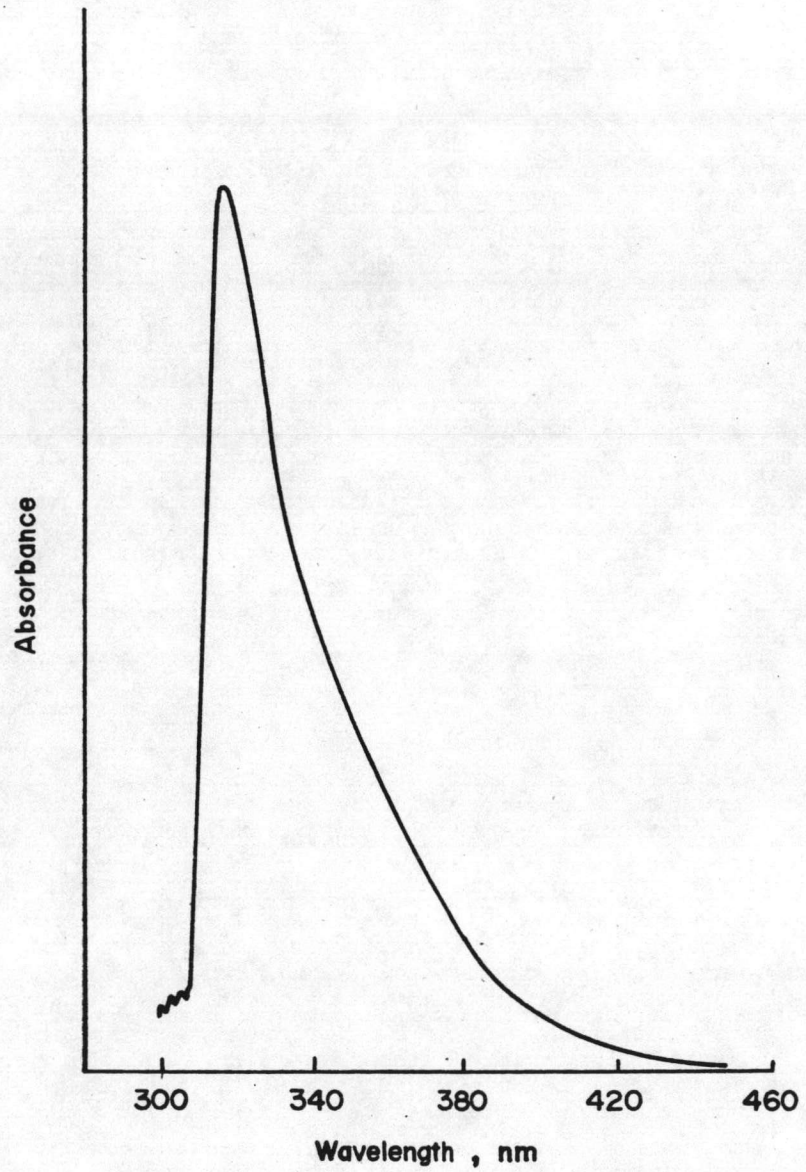

Figure 3. Absorption spectrum of phenylephrine – fluorescamine derivative.

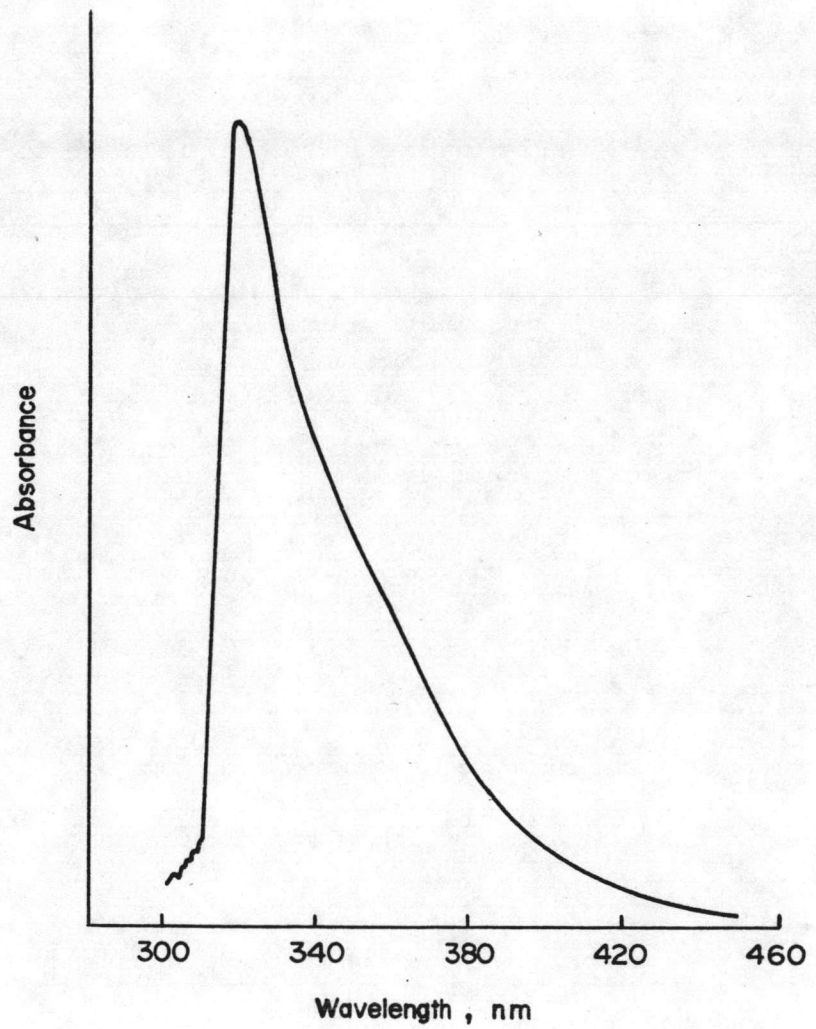

Figure 4. Absorption spectrum of epinephrine – fluorescamine derivative.

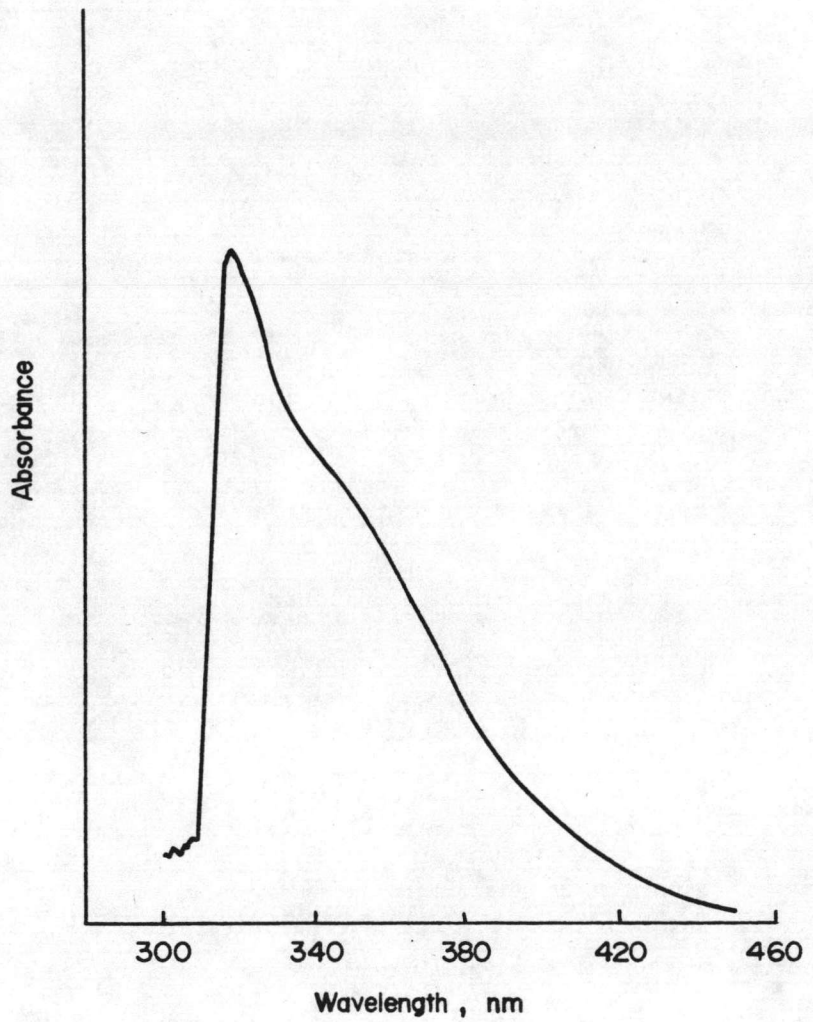

Figure 5. Absorption spectrum of metoprolol-fluorescamine derivative.

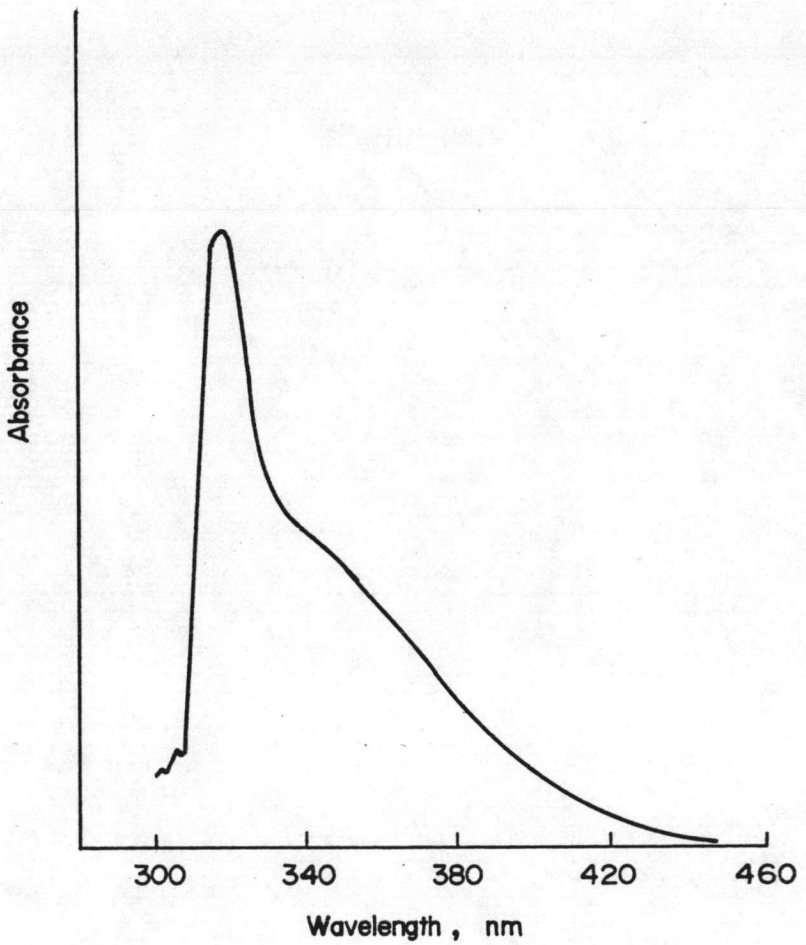

Figure 6. Absorption spectrum of propranolol - fluorescamine derivative.

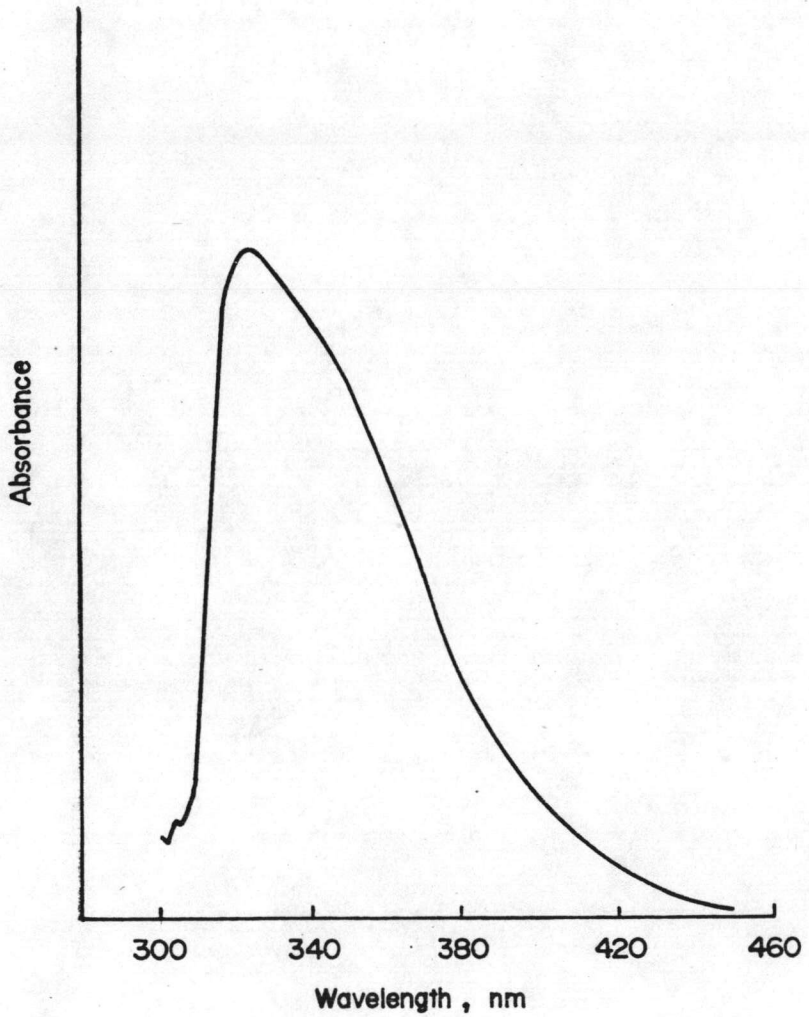

Figure 7. Absorption spectrum of piperazine - fluorescamine derivative.

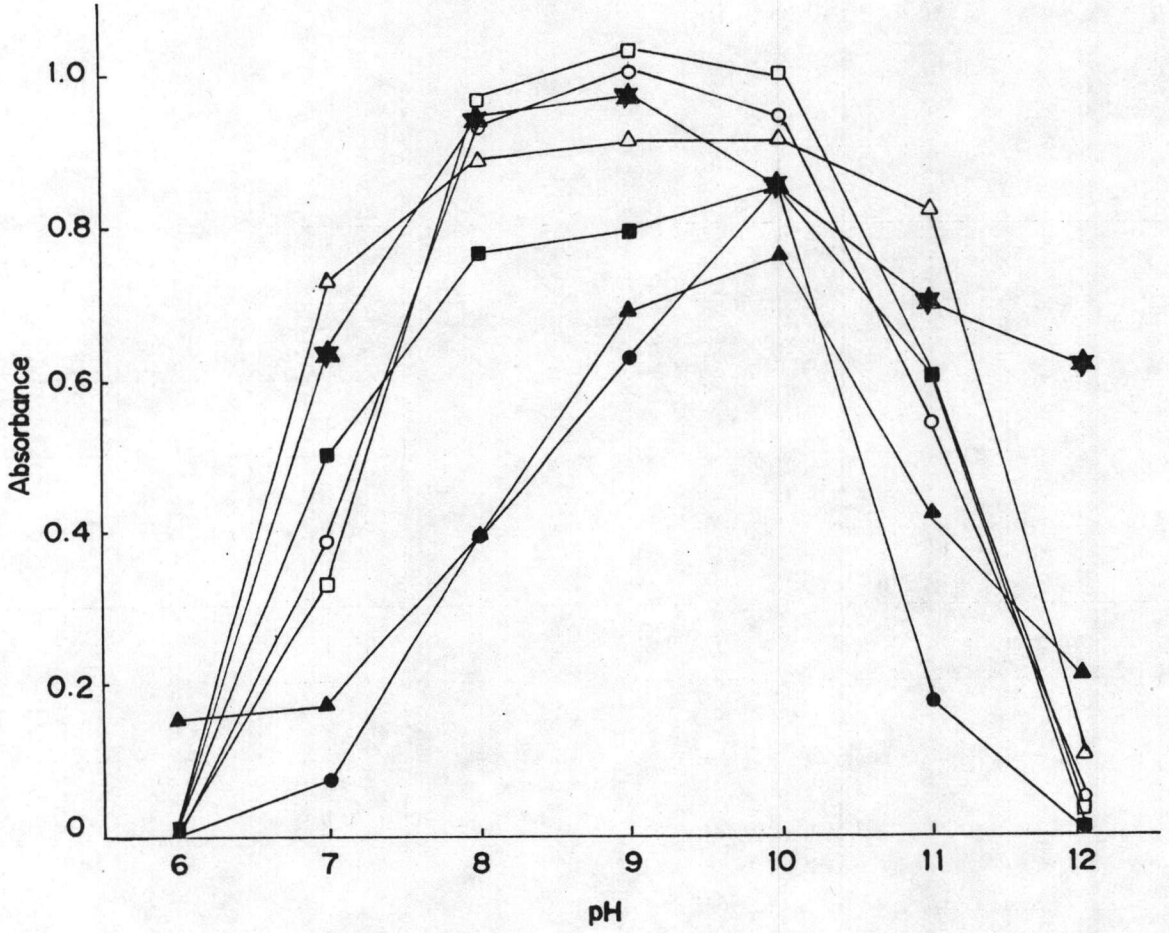

Figure 8. Effect of pH on the formation of secondary amine drug-fluorescamine derivatives.

Key : ○ , ephedrine ; □ , pseudoephedrine ; △ , phenylephrine ;

★ , epinephrine ; ● , metoprolol ; ▲ , propranolol ; ■ , piperazine.

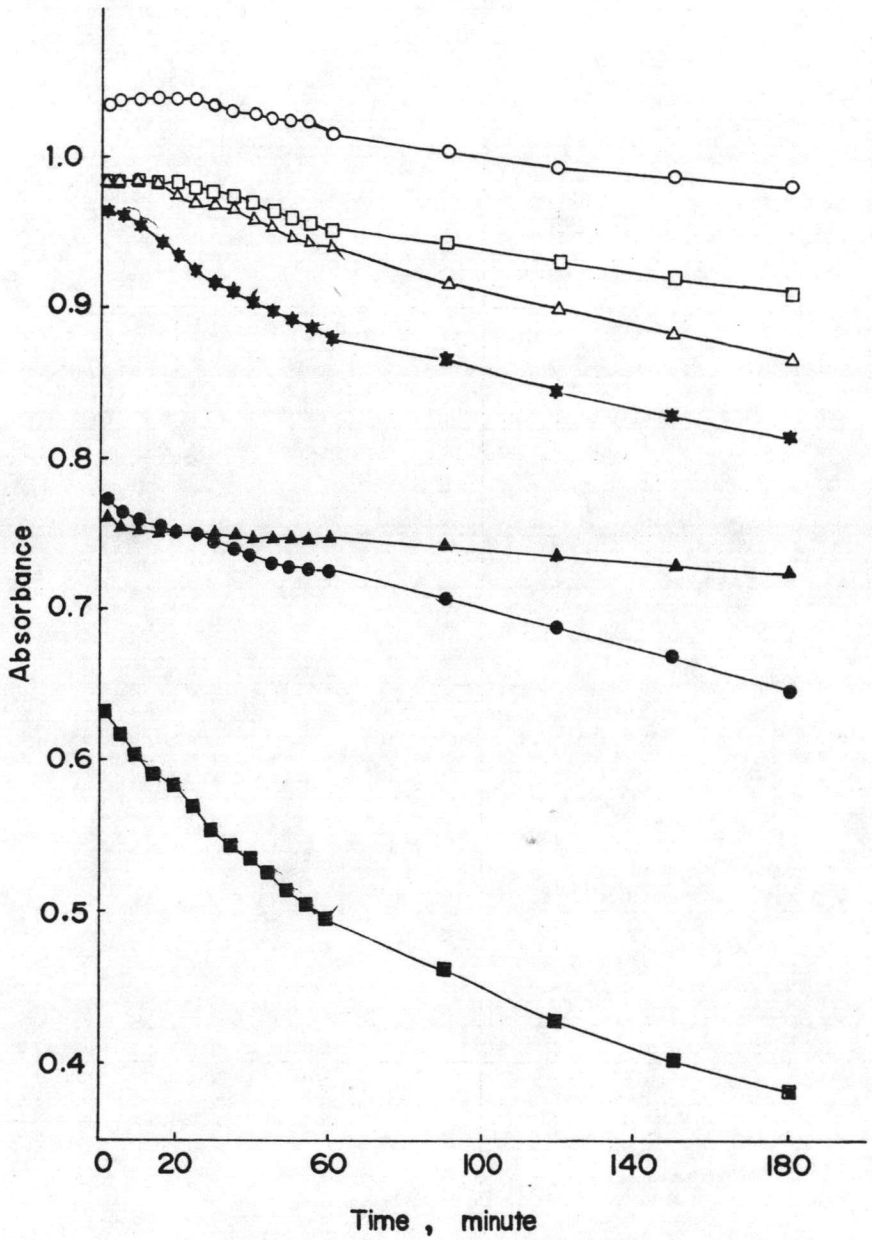

Figure 9. Effect of time on absorbance of secondary amine drug-fluorescamine derivatives.

Key : ○, ephedrine ; □, pseudoephedrine ; △, phenylephrine ; ✱, epinephrine ; ●, metoprolol ; ▲, propranolol ; ■, piperazine.

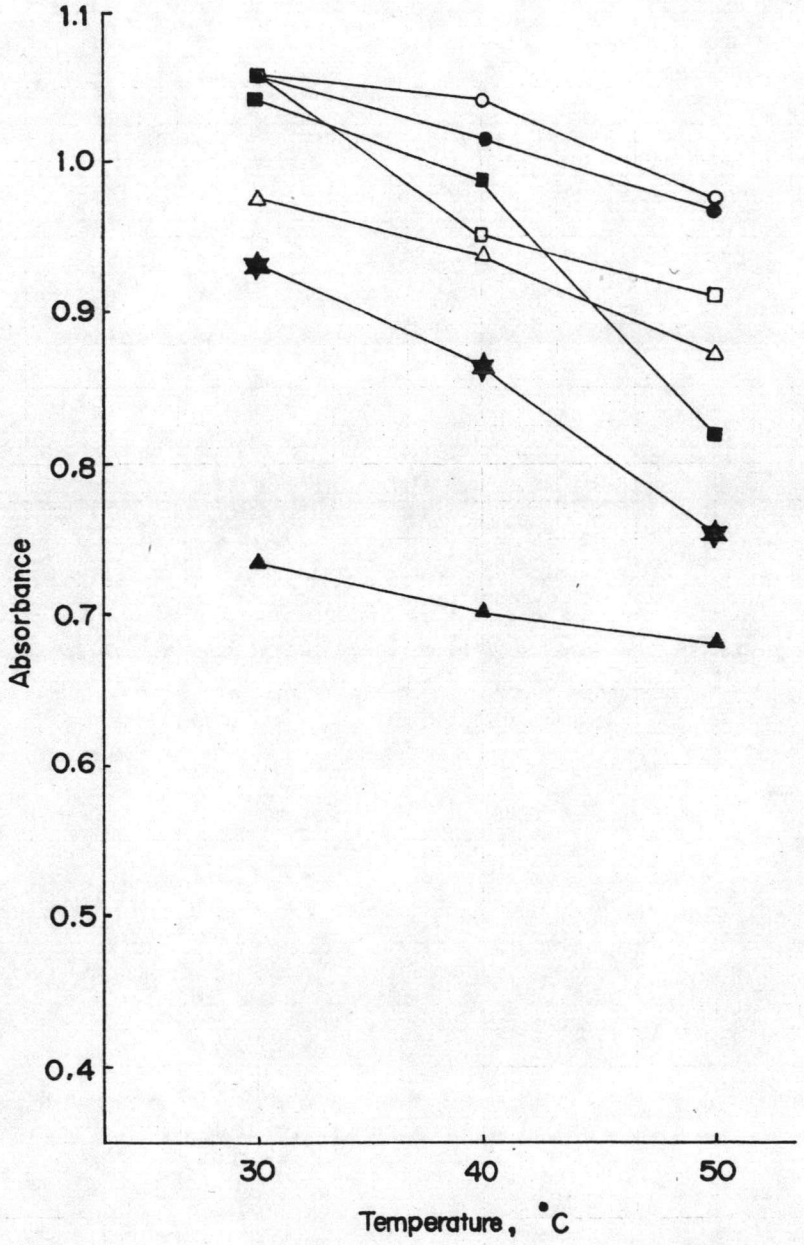

Figure 10. Effect of temperature on absorbance of secondary amine drug-fluorescamine derivatives.

Key : ○, ephedrine ; □, pseudoephedrine ; △, phenylephrine ; ★, epinephrine ; ●, metoprolol ; ▲, propranolol ; ■, piperazine.

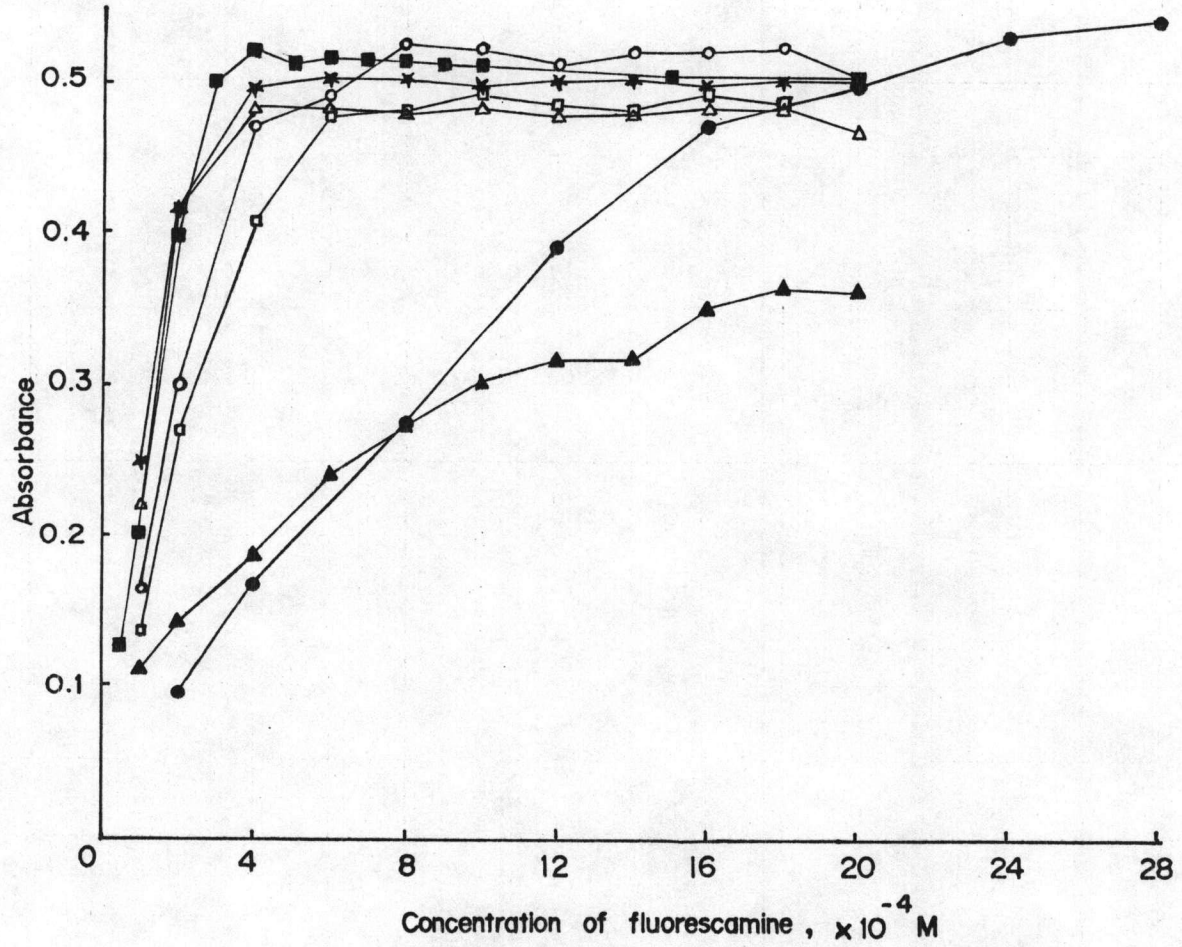

Figure 11. Effect of fluorescamine concentration on absorbance of secondary amine drug - fluorescamine derivatives.

Key : ○, ephedrine ; □ , pseudoephedrine ; △, phenylephrine ; ★, epinephrine ; ● , metoprolol ;

▲, propranolol ; ■ , piperazine.

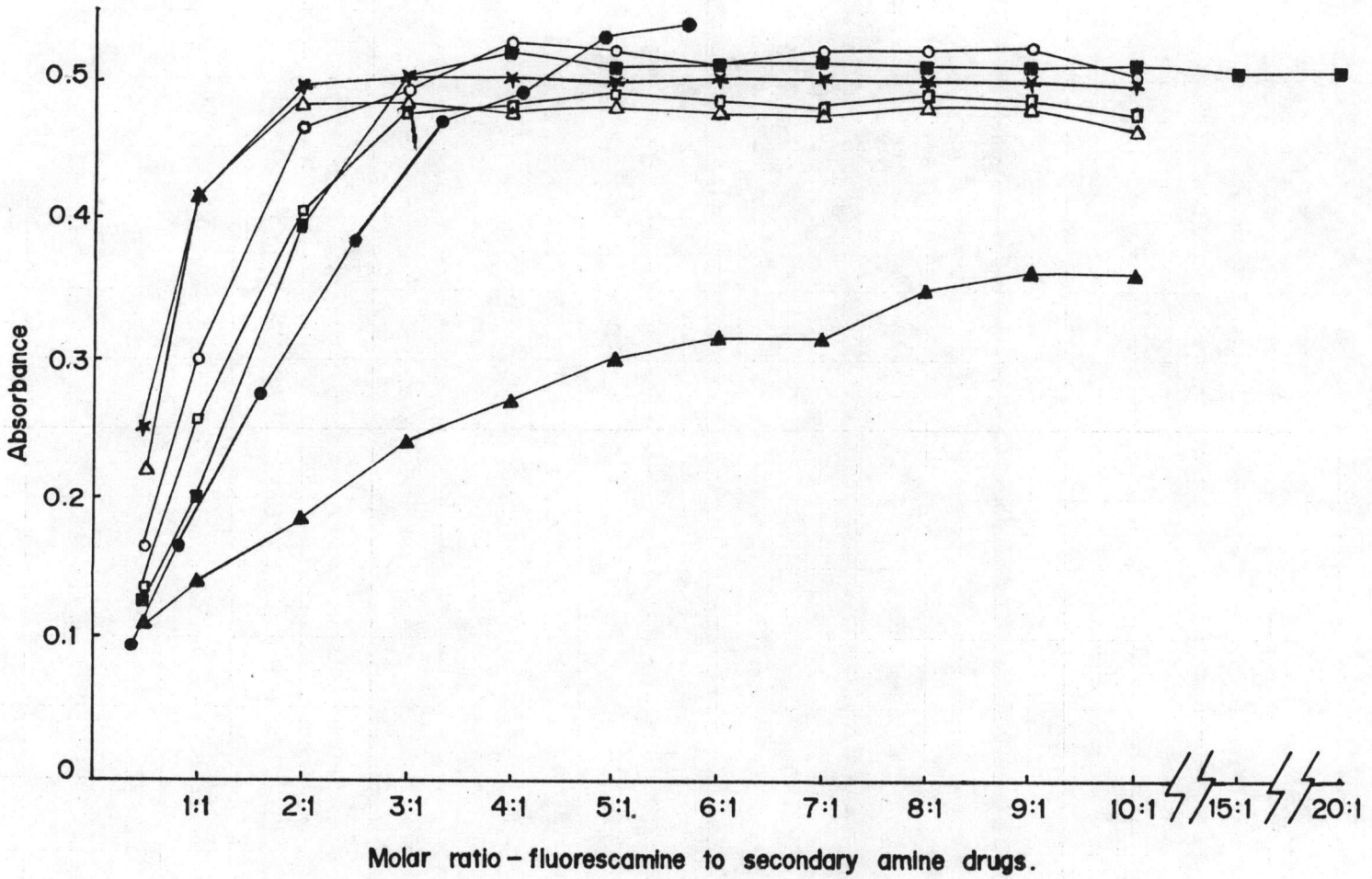

Figure 12. Mole ratio curve obtained from secondary amine drug - fluorescamine derivatives.

Key : ○, ephedrine ; □, pseudoephedrine ; △, phenylephrine ; ★, epinephrine ; ●, metoprolol ; ▲, propranolol ; ■, piperazine

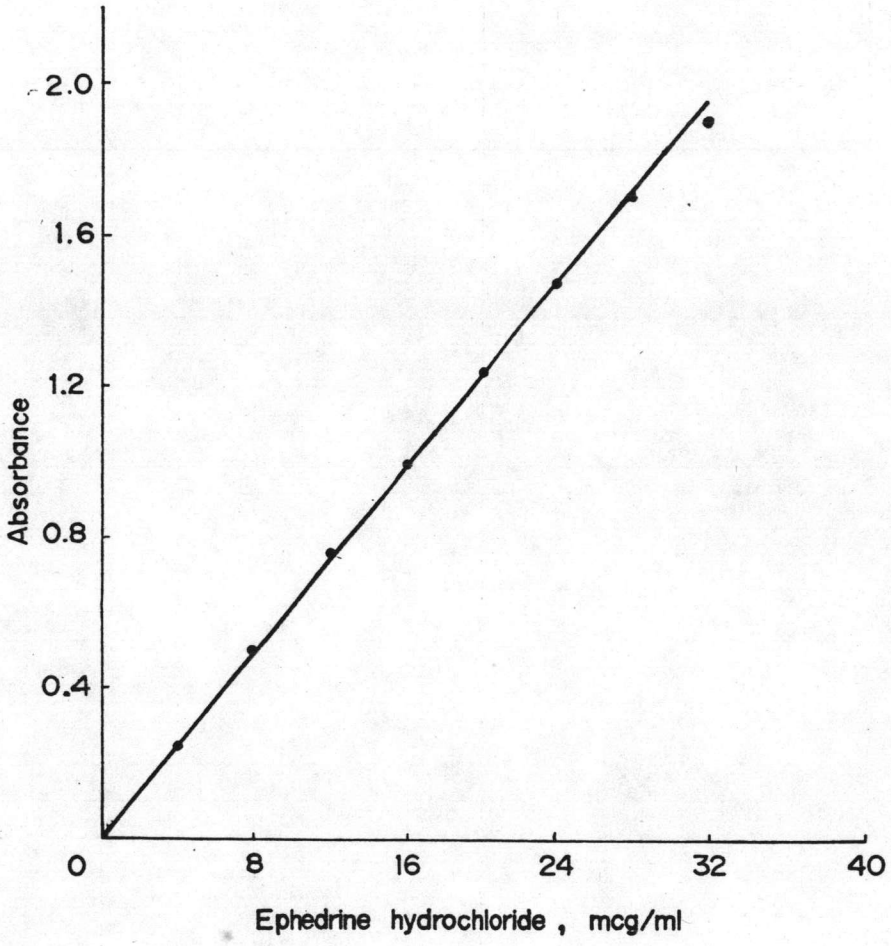

Figure 13. Calibration curve of ephedrine hydrochloride with fluorescamine.

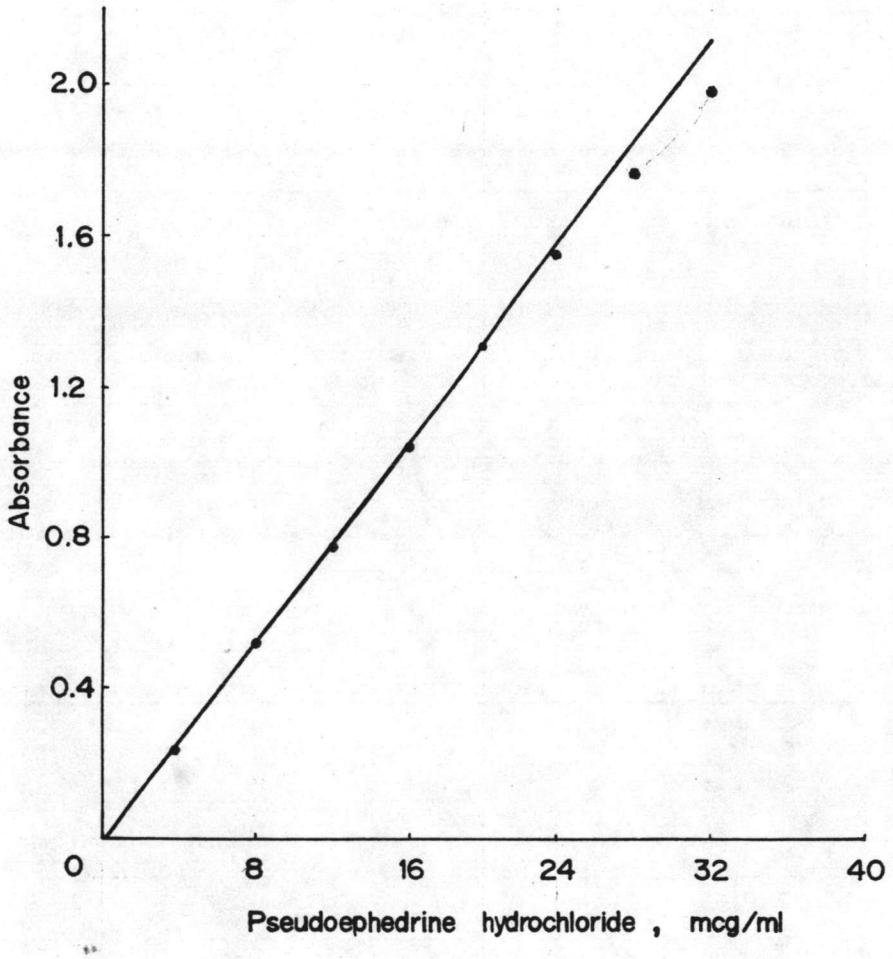

Figure 14. Calibration curve of pseudoephedrine hydrochloride with fluorescamine.

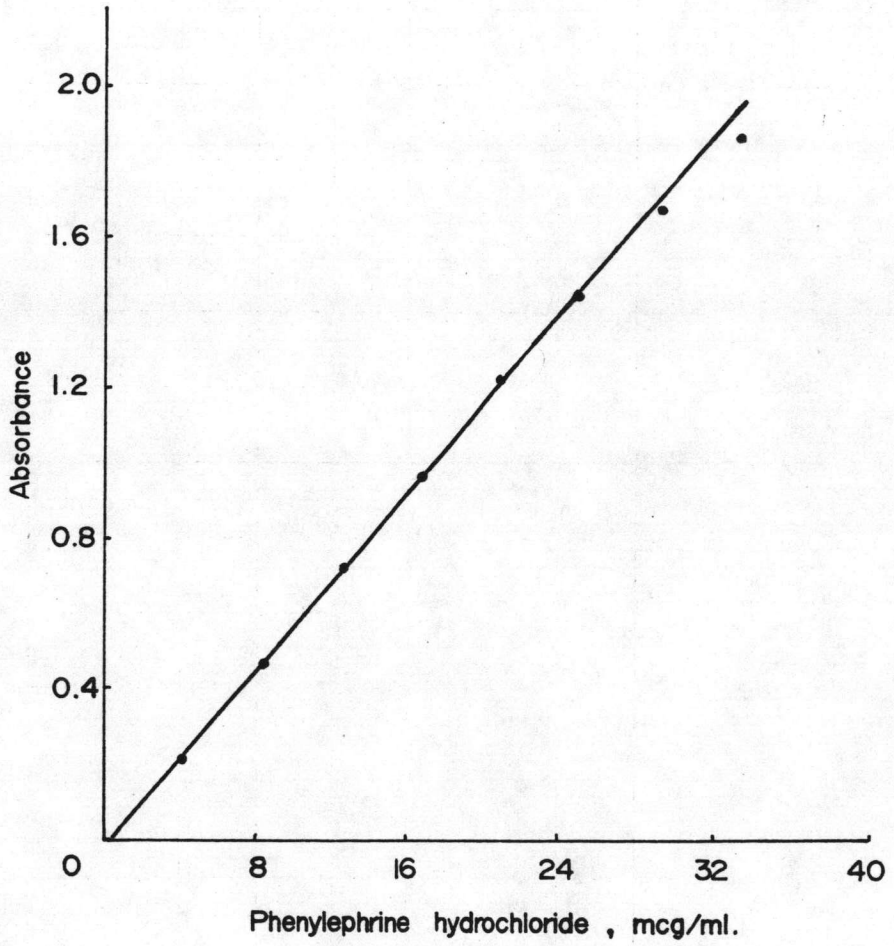

Figure 15. Calibration curve of phenylephrine hydrochloride with fluorescamine.

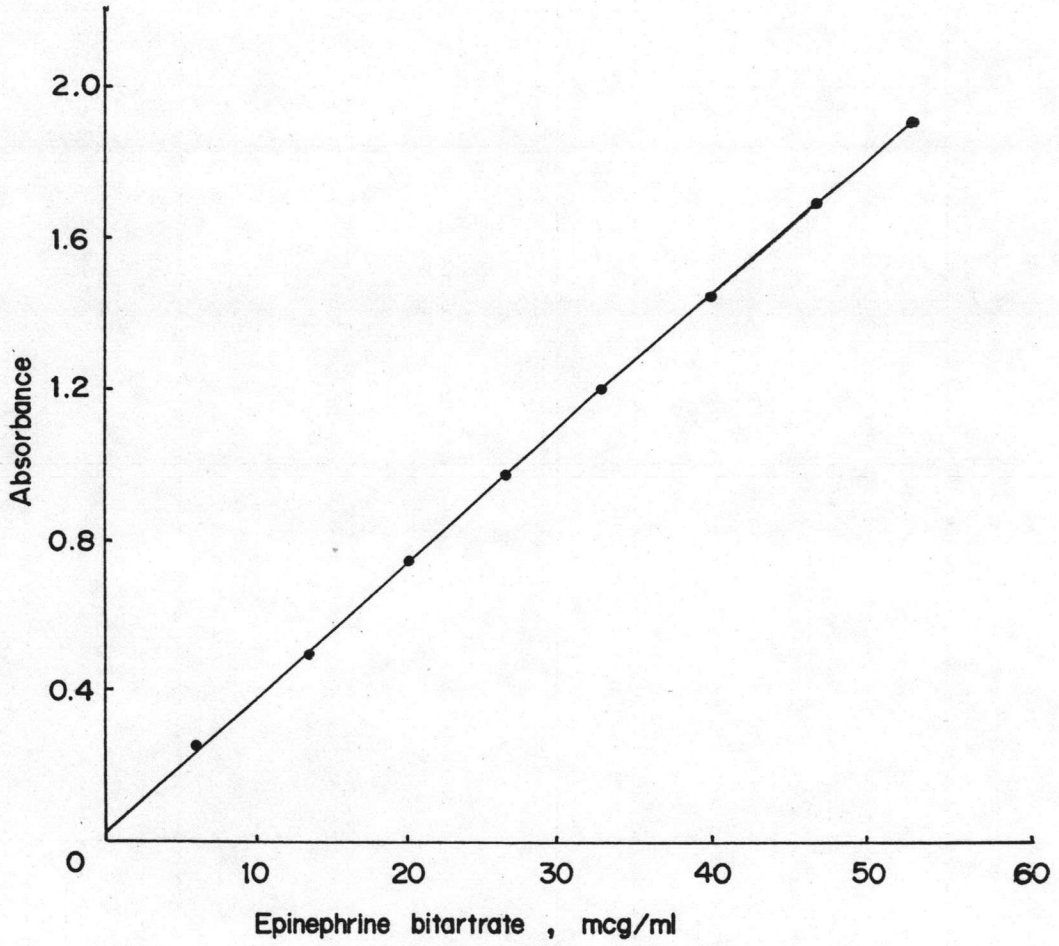

Figure 16. Calibration curve of epinephrine bitartrate with fluorescamine.

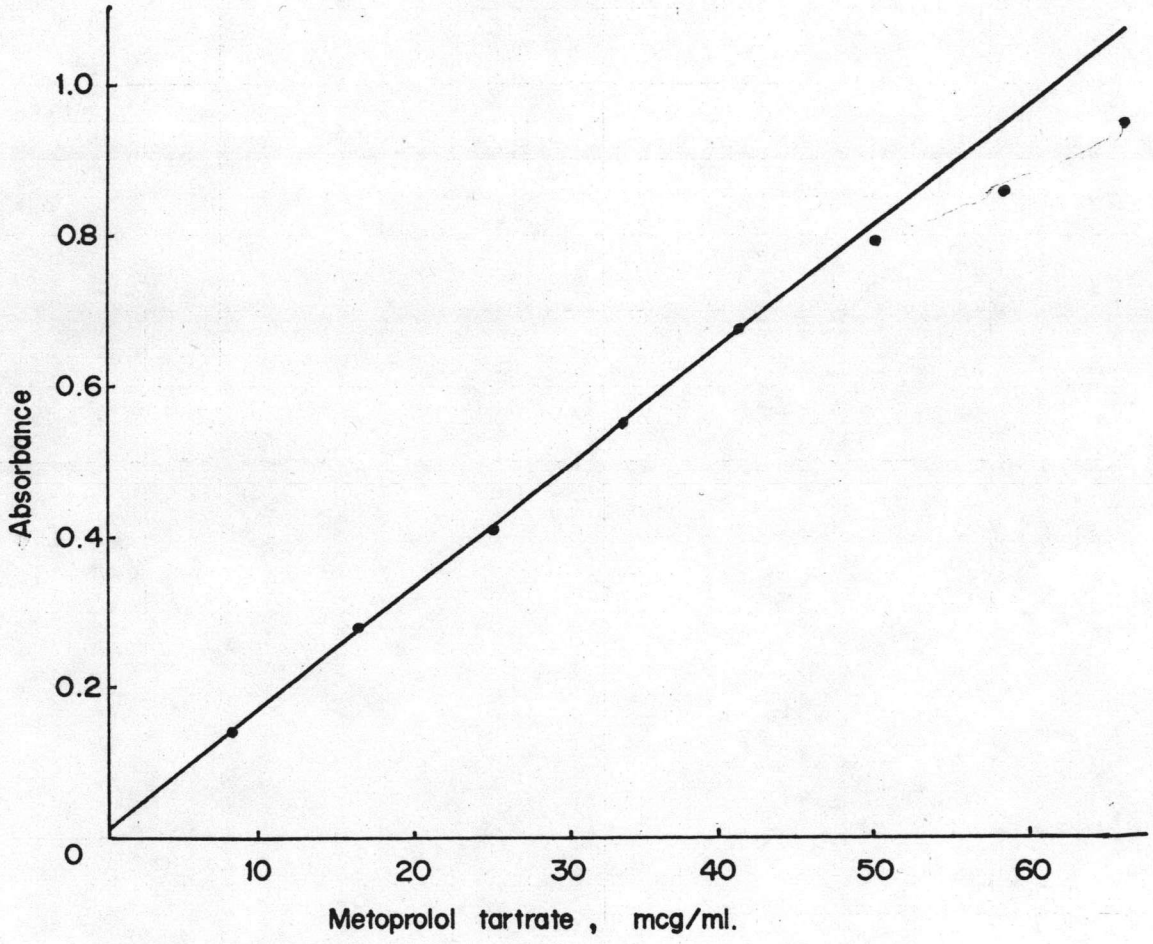

Figure 17. Calibration curve of metoprolol tartrate with fluorescamine.

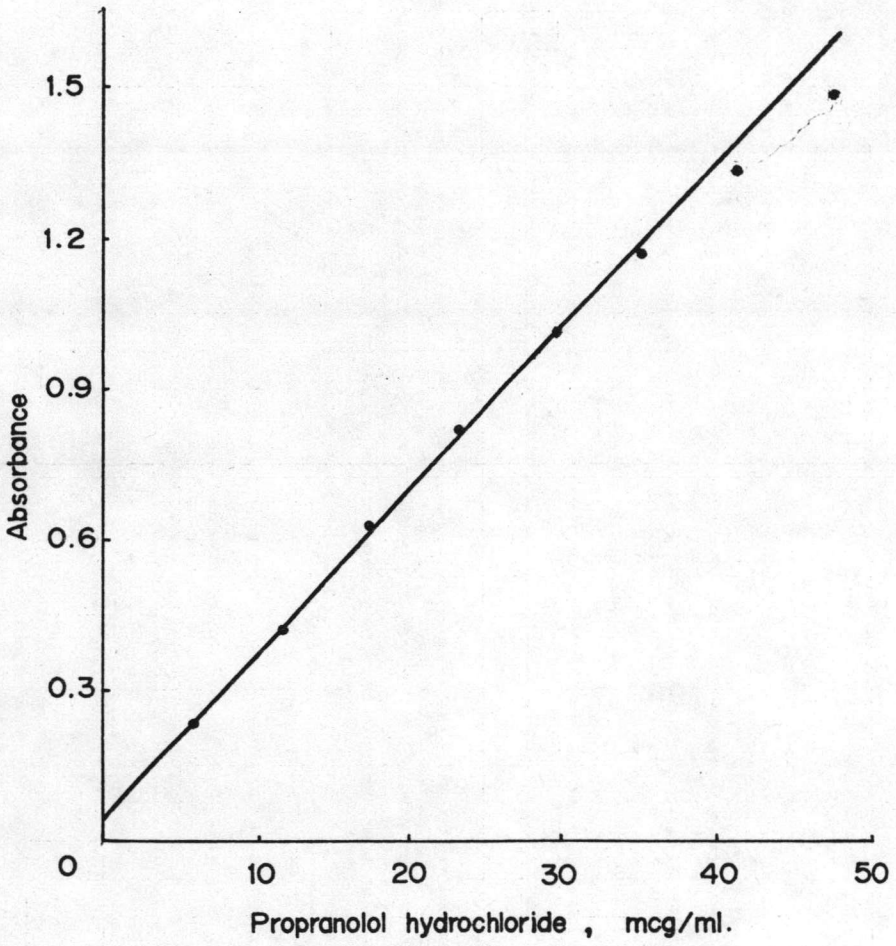

Figure 18. Calibration curve of propranolol hydrochloride with fluorescamine.

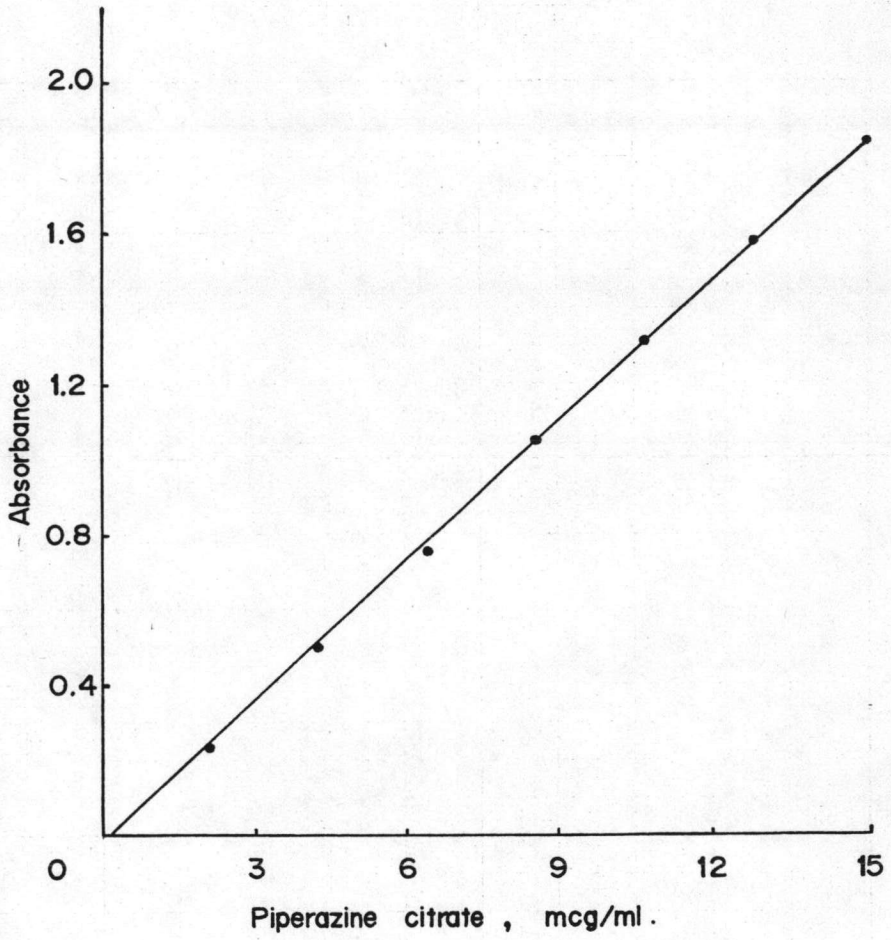

Figure 19. Calibration curve of piperazine citrate with fluorescamine.

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