CHAPTER II

ANALYTICAL METHODS

The drugs selected for the present study are official in many of the pharmacopoeias and assay procedures for these drugs are also mentioned therein. A brief review of estimation procedures of relevant drugs is presented here.

A. TETRACYCLINE HYDROCHLORIDE

Probably tetracycline hydrochloride is one of those drugs for which a number of analytical methods have been developed of which a brief account is presented below.

Spectrophotometric Methods : When tetracycline is dissolved in aqueous sodium hydroxide solution a stable yellow colour is produced with absorption maximum at 380 nm (1).

Tetracycline develops a colour with an absorption maximum at about 465 nm, when heated with solution of zinc chloride (2). When heated at 40°C in aqueous solution with sodium carbonate, sodium tungstate, and hydrogen peroxide; tetracycline gives a red-violet colour at 530 nm (3). Other substances which cause colour formation with tetracycline are zirconium oxychloride (4), thorium nitrate (5,6), ferric chloride (7), sodium bisulphate (8), boric acid - sulphuric acid (9), ammonium molybdate (10), diphenylboronic acid (11), toulene-o-stibonic acid (12) and hydroxyammonium chloride (13).

Mural (14) has reported colorimetric determination of tetracycline by the indophenol reaction. Its direct estimation in 0.1N hydrochloric acid at absorption maximum at 353 nm has been reported in B.P. (15).

Non-Aqueous Methods : Non-aqueous titration of tetracycline and some of its commercial preparations was reported by Yokoyama and Chatten (16) in 1958. Sample was dissolved in nitromethane, fumaric acid and benzene and titrated with perchloric acid in 1-4'-dioxan either potentiometrically or visually to green end point with a mixed indicator (methylene blue and quinoldine red in anhydrous methanol). Ellert et al. (17) described another non-aqueous method in which sample is dissolved in acetic anhydride and potentiometrically titrated with perchloric acid in 1-4'-dioxan. In a different method reported by Regosz (18), a sample of tetracycline hydrochloride and its derivatives were dissolved in 1 ml fumaric acid and diluted with acetic anhydride, acetic acid, acetic acid-benzene (3:1), propionic acid, nitromethane, or cyclohexane and 5% mercuric acetate solution in acetic acid was added. The resulting solution is titrated potentiometrically with perchloric acid in 1-4'-dioxan. Regosz in other series of publications (19-21) has reported other methods utilizing non-aqueous titrations.

Regosz et al. (22) have described a non-aqueous method with fluorimetric detection of the end point and by fluorimetry at 530 to 550 nm.

Microbiological Methods : The microbiological procedures used for the determination of tetracycline hydrochloride potency in body fluids, bulk products, and pharmaceutical formulations can be divided into two procedures, agar diffusion plate method (23-28) and turbidimetric method (29-31).

Duncombe (32) has described an autoanalyzer instrument system for the microbiological assay of tetracyclines. It was based on measurement of carbon dioxide resulting from free respiration of the test organism during fixed incubation time and the depression of respiration by graded concentration of antibiotic.

Complexometric Methods : Stahlavska et al. (33) have reported precipitation of tetracycline as their complex with nickel, the precipitate was then decomposed and the Ni^{2+} determined complexometrically.

Polarographic Methods : Hetman (34) has reported investigation on polarographic behaviour of tetracycline in 1963. A.C. polarography of tetracycline was reported by Caples et al. (35) in 1965. A solution containing 16 to 60 µg of antibiotic per ml in 0.2 M phosphate buffer of pH 4.1 was prepared, A.C. polarogram from 1.2 to 1.8 V vs. the mercury pool was recorded and the diffusion current at 1.65 Amps was measured. A.C. polarography for tetracycline analysis was again reported by Olliff and Chatten (36) in 1978.

Oscillopolarographic techniques in determination of tetracyclines have been reported by Faith (37) and Parrak et al. (38).

Chromatographic methods : Several paper chromatographic procedures have been developed for the separation of the tetracyclines (39). Fischbach and Lavine (40) have reported identification and separation of tetracyclines by paper chromatography. Kelley and Buyske (41) have reported paper chromatography on Whatman No. 1 paper treated with 0.01 M EDTA. Novelli et al. (42) have reported circular paper chromatography for quantitative determination of tetracycline. Antimony trichloride as a colour reagent in paper chromatography for determination of tetracyclines was used by Ozsoz (43).

Addison and Clark (44) have reported determination of tetracycline by ion exchange paper chromatography.

Thin layer chromatography has been used for both qualitative and quantitative analysis of tetracyclines. Quantitative determination of tetracycline hydrochloride by TLC has been reported by Simmons et al. (45) in 1969. After TLC, densitometry (46,47), UV light (48), fluorimetry (49,50) have also been used for quantitative estimation of tetracyclines and their impurities.

Column chromatography (51-54) has also been used for determination of tetracycline and its degradation products.

Fluorimetric Methods : The natural fluorescence of tetracycline and its derivatives has been used extensively to determine these compounds in pharmaceutical dosage forms as well as in biological materials. In alkaline solution tetracycline shows a yellow fluorescence (55). Kohn (56) showed that the fluorescent complex formed by tetracycline with calcium ions and barbitol can be measured spectrofluorimetrically. For the determination of tetracycline in blood and urine, use was made of its yellow fluorescence in the U.V. light (57). Hayes and Dubuy (58) have described a simple method of quantitative estimation of tetracycline antibiotics. Kelley et al. (59) and Hall (60) have suggested a fluorimetric method based on the formation of the aluminium chelate of tetracyclines. Kelley and Hoyt (61) have also suggested a fluorimetric determination of tetracyclines in mixture.

A study of the interaction of tetracycline with beryllium and fluorescence of formed complex has been reported by Alykova (62). Quantitative analysis of tetracyclines by direct fluorimetry after thin layer chromatography have been reported by Ragazzi and Veronese (49,63) and Ovcharova(50). Fluorimetry after column chromatography has been used by

Christophersen et al. (54) for analysis of tetracycline in human plasma. Fluorimetric end point detection of non-aqueous titration of tetracycline, and fluorimetry have been reported by Regosz et al. (64). Vanden and Kroon (65) have reported fluorimetric determination of tetracycline in small samples of blood and urine.

High Performance Liquid Chromatography : Knox and Jurund (66) have reported a simple and rapid method for complete separation and accurate determination at the sub µg level of tetracycline and its major impurities. Sharma et al. (67-69) have reported reversed phase high performance liquid chromatography for determination of tetracycline in urine and plasma. Several other authors (70-79) have also reported high performance liquid chromatographic techniques for its determination.

Miscellaneous Methods : Mahgoub et al. (80) reported physico-chemical studies, including conductometric, spectrophotometric, and microanalytical determinations on uranyl-tetracycline complex. Determination of tetracycline using UO_2^{++} was reported. Faraj and Ali (81) have reported radioimmunoassay for tetracycline. Bromination method for determination of tetracycline has been reported by few workers (82,83). Direct analysis of tetracycline in urine by circular dichroism spectropolarimetry has been reported by Bowen and Purdie (84).

B. HYDRALAZINE HYDROCHLORIDE

Several methods have been reported for estimation of hydralazine hydrochloride in pharmaceutical dosage forms, biological fluids and raw material from time to time. A brief review is given below.

Spectrophotometric methods : Solomonova et al. (85) determined hydralazine in tablets by a direct ultraviolet absorption method.

A frequently reported spectrophotometric technique for the determination of hydralazine is based on reactions with aromatic aldehydes to form hydrazones with absorption in the visible region. Luk'yanchikova et al. (86) used p-nitrobenzaldehyde. Wesley-Hadzija and Abaffy (87) and Ruggieri (88) used dimethylaminobenzaldehyde. Luk'yanchikova (89,90) used cinnamaldehyde; Schulert (91) used p-hydroxybenzaldehyde; and Zak et al. (92) used p-methoxybenzaldehyde, salicylaldehyde, 3,4,5-trimethoxybenzaldehyde, and 1-naphthaldehyde.

Perry (93) and Grabowicz and Brulinska (94) used ninhydrin to obtain a solution with an absorption maximum at 450 nm.

Ellert and Modras (95) treated hydralazine with ferrous ion in alkaline solution, and measured the colour produced at 540 nm. Ruggieri (88) reported a colorimetric test using

2-naphthoquinone sulphonate sodium to form a rose-violet colour. Urbanyi and O'Connell(96,97) developed an automatic colorimetric method using blue tetrazolium for the analysis of hydralazine in the presence of reserpine and hydrochlorothiazide.

USP XX (98) describes use of ferric ammonium sulphate and 1,10-phenanthroline in development of colour with an absorption maximum at about 510 nm for analysis of hydralazine hydrochloride in reserpine, hydralazine hydrochloride and chlorothiazide tablets.

Titration Methods : Ruggieri (88) reported titrating hydralazine with perchloric acid.

Ruzhentseva et al. (99) converted hydralazine to ammonia by heating with zinc in sulphuric acid, forming three moles of ammonia per mole of hydralazine. The mixture was made alkaline and the ammonia was distilled into boric acid solution, which was then titrated.

Sandri (100) tested three methods with good results. One was titration with potassium bromide and hydrochloric acid using a starch-iodine end point. Another was addition of periodic acid-potassium iodide with sodium thiosulphate back titration. The third was addition of sodium hydroxide and a concentrated solution of potassium ferrocyanide, then acidifying and titrating with potassium permanganate solution.

Perel'man and Evstratova (101) titrated hydralazine in dimethylformamide solution to a potentiometric end point with sodium methoxide solution.

Artamanov et al. (102) determined hydralazine hydrochloride by conductometric titration with sodium hydroxide solution. Goryacheva and Prikhodkina (103) titrated hydralazine from tablets with N-bromosuccinamide solution using methyl red as indicator.

Soliman and Belal investigated argentometric (104) and mercurimetric (105) methods. USP XIX recommends that hydralazine be determined in the raw material, tablet and injection by potassium iodide titration in strongly acid solution, using chloroform to detect the presence of iodine (106).

Gasometric Methods : Mckennis and Yard (107) studied the nitrogen evolution from a series of hydrazino compounds when treated with 0.02 M potassium iodate in 0.2 N sulphuric acid in a Warburg apparatus at 37°C. Viala (108) determined hydralazine in solutions or tablets measuring the nitrogen freed from the hydrazine group, using potassium permanganate in sulphuric acid, or iodine in sodium bicarbonate solution.

Polarographic Methods : Polarographic studies of hydralazine and related compounds were reported by Giovanoli-Jakubezak et al. (109) and by Modras (110). The reduction proceeds in

2-electron stages with the formation of the tetrahydro derivative.

Chromatographic Methods : Ruggieri (88) and McIsaac and Kanda (111) describe chromatography on Whatman No. 1 paper with butanol : acetic acid : water (4:1:5) as the solvent. The R_f value for hydralazine was 0.90. Several investigators (112-114) have used alkaline systems for paper chromatography of hydralazine and its metabolites, but according to Lesser et al. (115) alkaline chromatography may not be suitable for hydralazine itself.

Stohs and Scratchley (116) investigated several thin layer chromatography for thiazide diuretics and antihypertensive drugs, using silica gel G and a variety of detection reagents-methylethyl ketone : n-hexane (1:1) - 0.72; methyl ethyl ketone : n-hexane (2:1) - 0.62; methyl ethyl ketone : n-hexane (3:1) - 0.00; chloroform : acetone : triethanolamine (50:50:1.5) - 0.68, are few solvent systems used for thin layer chromatography and R_f value of the hydralazine in that system.

Zak et al. (92) used N-hydrochloric acid : methanol : ascorbic acid (44:6:1) on silica gel and found R_f value of 0.53 for hydralazine. Lesser et al. (115) used cellulose sheets and silica gel with fluorescent indicator.

Fluorimetric Methods : Naik et al. (117) extracted hydralazine hydrochloride from tablets with 50% aqueous methanol and extract was treated with sulphuric acid concentrated to obtain fluorescence at 353 nm with excitation at 320 nm. Injections were analysed similarly.

Gas Liquid Chromatographic Methods : Jack et al. (118) determined hydralazine in plasma. The sample was treated with nitrous acid, which reacts with hydralazine to form tetrazolo (1,5a) - phthalazine. The derivative was extracted with benzene and determined by gas chromatography.

The same procedure, or modification of it was used by Zak et al. (119), Talseth (120-123), and Haegele et al. (124) for metabolic studies.

Smith et al. (125) determined hydralazine in tablets. An aqueous extract of the tablets was treated with 2,4-pentanedione, forming 1-(3,5-dimethyl pyrazole) phthalazine. The method was applied to stability studies of tablets subjected to elevated temperatures where tablets could not be analysed by the USP method.

Several other workers (126-130) have also reported gas chromatography for determination of hydralazine and its metabolites.

High Pressure Liquid Chromatographic Methods : Smith et al. (131,132) analysed hydralazine in a drug mixture containing hydralazine, hydrochlorothiazide, and an impurity derived from latter. The column was 1 m x 2.1 mm (I.D.) stainless steel, packed with a strong anion exchanger on 30 µm Zipax(R). The mobile phase was pH 9.2 borate buffer containing 0.005 M sodium sulphate (5% methanol), at 1.7 ml per minute. Detection was by ultraviolet absorption at 254 nm.

Honigberg et al. (133) tested reversed phase chromatography for separation of a number of drugs, including hydralazine. The columns contained either octadecyltrichlorosilane or diphenyldichlorosilane bonder to 37 to 50 µm pellicular silica packing of the various mobile phases tested, the best for separating hydralazine, hydrochlorothiazide, and reserpine was acetonitrile : 0.1% ammonium acetate (20:80), pH 7.35. The columns were 1.22 mm x 2.3 mm (I.D.) and the flow rate was 1.4 ml per minute. Detection was by ultraviolet absorption at 254 nm. Several other investigators (130,134-137) have also reported the analysis of hydralazine as well as its metabolites by HPLC.

PREPARATION OF CALIBRATION CURVES

The following raw materials were used in the preparation of calibration curves of tetracycline hydrochloride and hydralazine hydrochloride.

Raw Materials :

Tetracycline hydrochloride oral grade (Synbiotics Ltd., India), Hydralazine hydrochloride (Sarabhai Chemicals, India), Hydrochloric acid A.R. (Sarabhai M. Chemicals, India), Uranium acetate (British Drug House, U.K.), β-thiopropionic acid (Fluka AG. Chem. Fabrik, CH-9470 Buchs.), 1-4'-Dioxane pure (Sarabhai M. Chemicals, India), 1-10-phenanthroline (Loba Chemicals, India), Ferric ammonium sulphate C.P. (Sarabhai M. Chemicals, India), Sodium acetate trihydrate (Sarabhai M. Chemicals, India), Glacial acetic acid A.R.(Sarabhai M. Chemicals, India), p-methoxybenzaldehyde (Koch Light Laboratories Ltd., U.K.), Sodium hydroxide A.R. (S.D. Fine Chem. Pvt. Ltd., India), Benzene G.R. (S.D.Fine Chem. Pvt. Ltd., India), Methanol pure (Sarabhai M. Chemicals, India), Sodium chloride G.R.(S.D.Fine Chem. Pvt. Ltd., India), Potassium phosphate monobasic L.R.(Sarabhai M.Chemicals, India), Shellac (Chemicals Supply Corpn., India), Polyvinyl pyrrolidone K30 (S.D.Fine Chem. Pvt. Ltd., India), Cellulose acetate phthalate 50 CP (Wilson Lab., India), Silica gel (E. Merck, West Germany), Succinic acid IP (Sarabhai M. Chemicals, India), Avicel (Cellulose Products of India, India), Ethyl cellulose 5% 100 CP (Aldrich Chemical Co., U.S.A.), Glyceryl monostearate (Croda Chemicals, U.K.), Glyceryl distearate (Croda Chemicals, U.K.), white bees wax (S.D. Fine Chem. Pvt. Ltd., India), Tween-80 (Hico products

Ltd., India), Propylene glycol L.R. (Sarabhai M. Chemicals, India), Dibutylphthalate (Riedel-Dehaen AG Seelze-Hannover), Triacetin (Riedel-Dehaen AG Seelze-Hannover), Eudragits (Rohm Pharma GmbH West Germany), Lactose (Chemicals Supply Corpn. India), Magnesium stearate (Chemicals Supply Corpn., India), Talc (Chemicals Supply Corpn., India).

I. TETRACYCLINE HYDROCHLORIDE

(a) In Vitro Evaluation :

British Pharmacopoieal(15) method of finding dissolution rate of tetracycline hydrochloride has been used for in vitro evaluation of various formulations.

(i) Reagents :

0.1N Hydrochloric acid solution :

9 ml of concentrated hydrochloric acid was taken in a 1 L volumetric flask and volume was made up with purified water.

(ii) Preparation of standard solutions :

500 mg of pure tetracycline hydrochloride was weighed accurately and dissolved in 100 ml of 0.1N hydrochloric acid. 10 ml of this solution was further diluted to 100 ml with 0.1N hydrochloric acid. Finally 1,2,3,4,5,6,7 and 8 ml of the stock solution were taken in eight 100 ml volumetric flasks and volume was made with 0.1N hydrochloric acid.

(iii) Procedure :

Absorbance of solution of each flask was measured at 355 nm on Hitachi-Perkin Elmer-139, UV and Visible Spectrophotometer, against 0.1N hydrochloric acid as blank. Results are shown in Table 2-1 and Figure 2-1.

(b) In vivo evaluation

Methods reported by Mahgoub et al. (80) and Hall (60) were utilized to develop the method of estimation of tetracycline hydrochloride in human urine for present investigation. The method is based on formation of yellow complex, uranyl-tetracycline, with an absorption maximum in visible region.

(i) Reagents :

β-thiopropionic acid solution :

0.1 ml of β-thiopropionic acid was taken in a 10 ml volumetric flask, volume was made up with purified water and filtered.

Uranium acetate solution :

50 mg of uranium acetate was dissolved in 50 ml of 1-4'-dioxane - purified water mixture (1:1) in a 50 ml volumetric flask and filtered.

0.1N hydrochloric acid solution :

9 ml of concentrated hydrochloric acid was taken in a 1 L volumetric flask and volume was made up with purified water.

(ii) Preparation of standard solutions :

100 mg of tetracycline hydrochloride was dissolved in 100 ml of 0.1N hydrochloric acid. 10 ml of this solution was taken in 100 ml volumetric flask and volume was made up with urine - 0.25, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 ml of this solution were taken in eight volumetric flasks and volume was made to 4 ml with urine in each case.

(iii) Procedure :

To each flask 1.0 ml of 1,4-dioxane, 2.0 ml of 0.1N hydrochloric acid and 0.5 ml of β-thiopropionic acid solution were added and flasks were heated at 100°C for 15 minutes in a waterbath. Solution was cooled to room temperature and 0.2 ml of uranium acetate solution was added to each flask. Finally volume was made to 10 ml with 1,4-dioxane-purified water mixture (1:1). The absorbance of each solution was measured at 430 nm on a Spectronic-20 spectrophotometer using plain urine treated similarly as a blank. Observations are given in Table 2-2 and the standard calibration curve is plotted in Figure 2-2.

TABLE 2-1.

ESTIMATION OF TETRACYCLINE HYDROCHLORIDE IN DISSOLUTION MEDIUM

S.No.	Concentration (µg/ml)	Absorbance
1	5	0.15
2	10	0.30
3	15	0.43
4	20	0.58
5	25	0.70
6	30	0.84
7	35	0.99
8	40	1.13

TABLE 2-2

ESTIMATION OF TETRACYCLINE HYDROCHLORIDE IN HUMAN URINE

S.No.	Concentration (µg/ml)	Absorbance
1	2.5	0.04
2	5.0	0.09
3	10.0	0.16
4	15.0	0.28
5	20.0	0.36
6	25.0	0.44
7	30.0	0.53
8	35.0	0.62

FIG. 2-1: CALIBRATION CURVE OF TETRACYCLINE HYDROCHLORIDE IN DISSOLUTION MEDIUM.

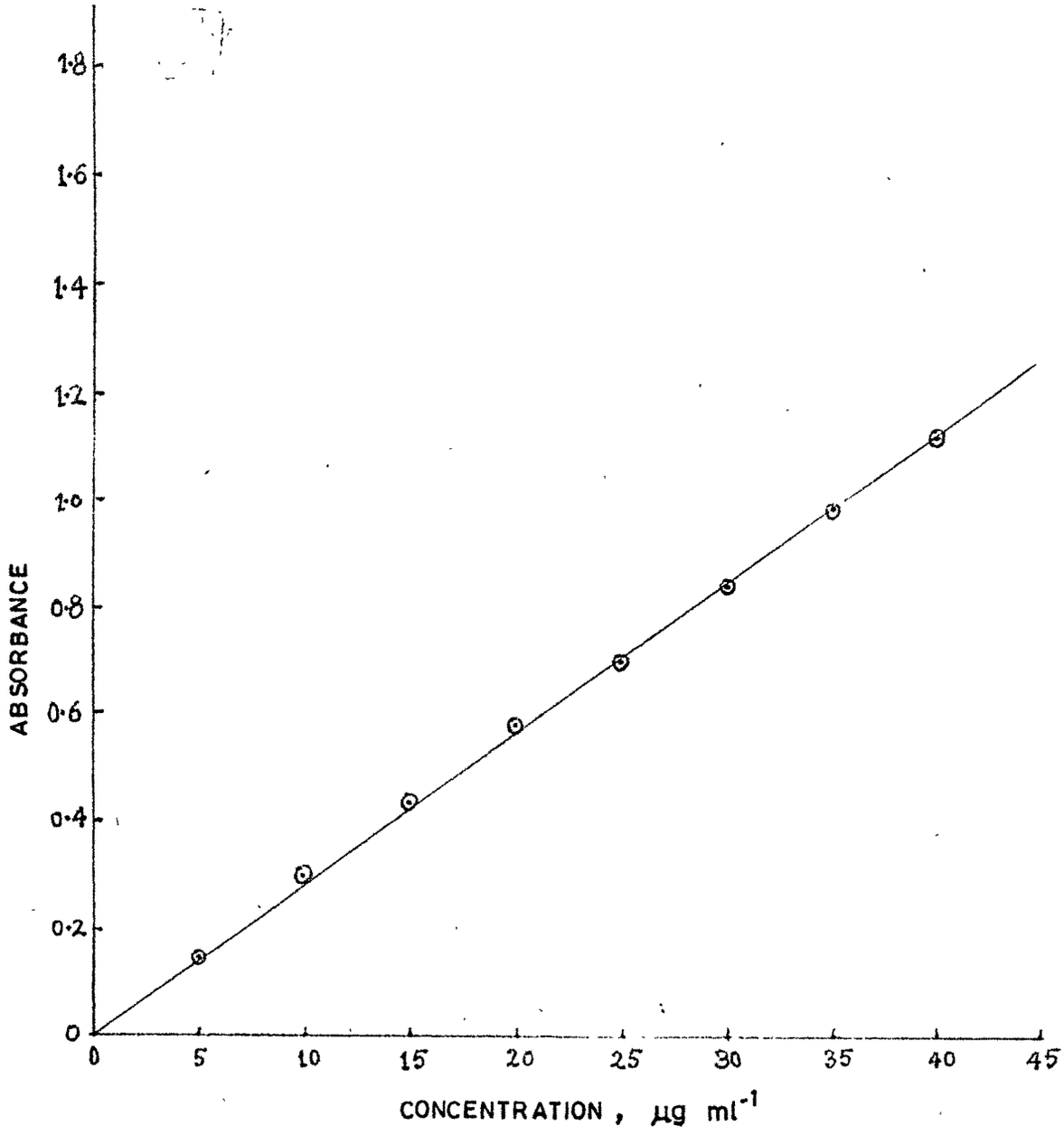
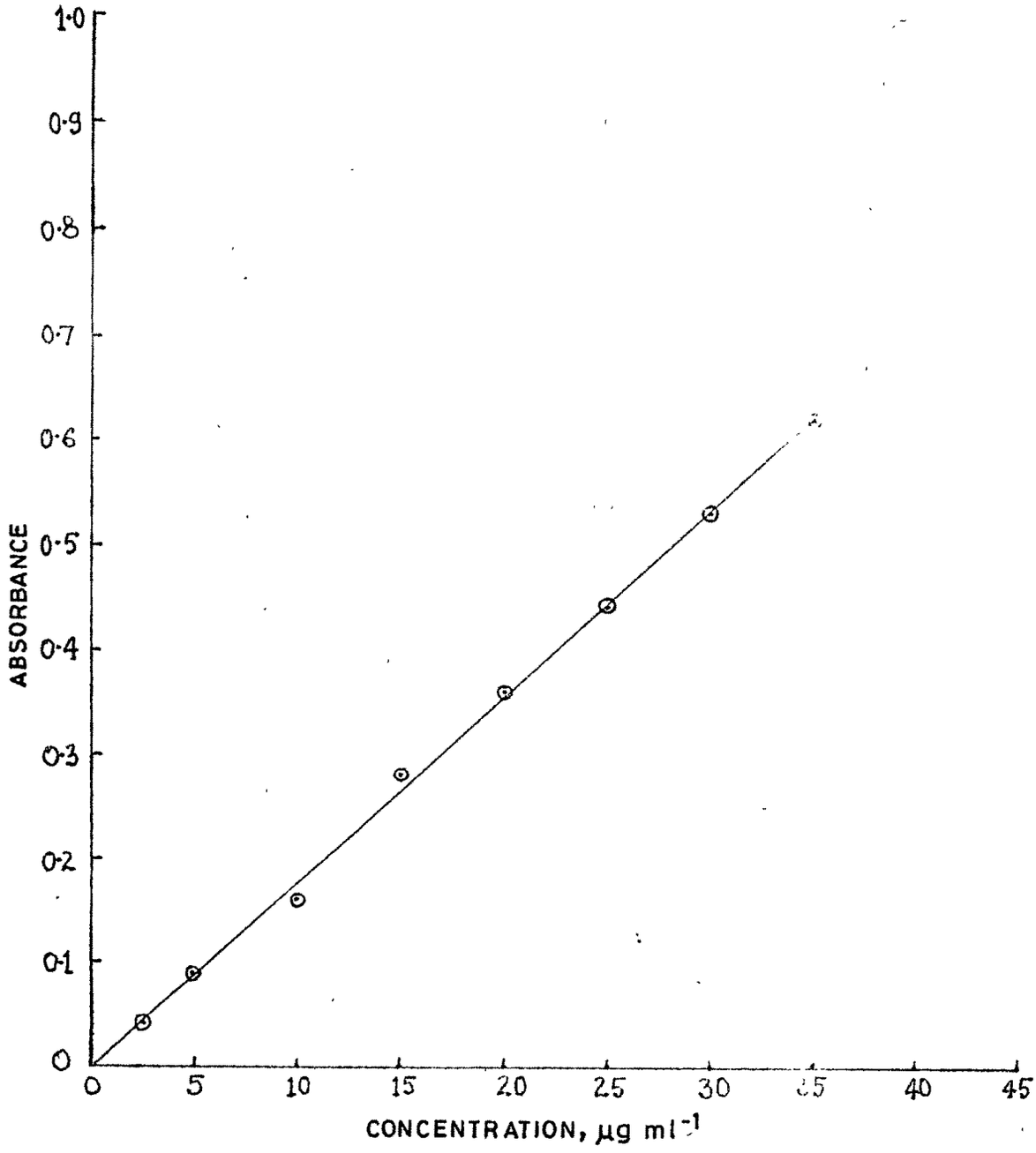


FIG. 2-2 : CALIBRATION CURVE OF TETRACYCLINE HYDROCHLORIDE IN HUMAN URINE



II. HYDRALAZINE HYDROCHLORIDE

(a) In vitro evaluation

USP XX (98) method of estimation of hydralazine hydrochloride in reserpine, hydralazine hydrochloride and chlorothiazide tablets was used for *in vitro* evaluation of hydralazine hydrochloride. The method is based on formation of complex of ferrous ions with 1,10-phenanthroline.

(i) Reagents :

Sodium acetate solution :

27.2 g of sodium acetate trihydrate was dissolved in 50 ml of purified water. The solution was cooled to room temperature and volume was made to 100 ml with purified water.

Ferric ammonium sulphate solution :

1.8 g of ferric ammonium sulphate was dissolved in 4 ml of dilute hydrochloric acid (8.3% v/v) and volume was made to 100 ml with purified water. Prepared solution was filtered and the clear solution was used. Fresh solution was used each time.

1,10-Phenanthroline solution :

300 mg of 1,10-phenanthroline was shaken with 100 ml purified water for 1 hour and filtered. Clear solution was used for analysis. Fresh solution was prepared each time.

(ii) Preparation of Standard solutions :

50 mg of hydralazine hydrochloride was accurately weighed and dissolved in a 100 ml volumetric flask and volume was made up with purified water. 10 ml of the solution was further diluted to 100 ml with purified water. 1,2,3,4,5,6,7 and 8 ml of the stock solution was taken in eight different 100 ml volumetric flasks.

(iii) Procedure :

To each flask 2.5 ml of acetic acid solution (12% v/v), 2.5 ml of sodium acetate solution, 1 ml of 1,10-phenanthroline solution and 0.5 ml ferric ammonium sulphate solution were added. The solutions were mixed and allowed to stand in dark for 30 minutes. Volume was made up with purified water. Absorbance of each solution was measured at maximum of 510 nm on a Spectronic-20 spectrophotometer against the blank. The observations are shown in Table 2-3 and the standard calibration curve is plotted in Figure 2-3.

(b) In vivo evaluation

The method reported by Zak et al. (92) was used for in vivo determination of hydralazine hydrochloride. Spectrophotometric technique for determination of hydralazine is based on reaction with p-methoxybenzaldehyde to form hydrazone with absorption in the visible region.

(i) Reagents :

p-methoxybenzaldehyde solution :

1 ml of p-methoxybenzaldehyde was taken in 100 ml flask and volume was made up with ethyl alcohol and filtered.

Ascorbic acid solution :

10 g of ascorbic acid was taken in 100 ml volumetric flask and dissolved in sufficient purified water and volume was made up with purified water. The solution was filtered and fresh solution was used whenever necessary.

5 N Sodium hydroxide solution :

20 g of sodium hydroxide was dissolved in purified water and volume was made up to 100 ml with purified water. The solution was filtered and prepared fresh each time.

Benzene-Methanol solution :

70 ml of methanol was mixed with 930 ml of benzene.

0.2 N, 0.4 N and 6.0 N Hydrochloric acid solutions :

1.8 ml, 3.6 ml and 54 ml concentrated hydrochloric acid was taken in three different 100 ml volumetric flasks and volume was made up with purified water.

(ii) Preparation of Standard solutions :

100 mg of hydralazine hydrochloride was taken in 500 ml volumetric flask and dissolved in purified water and volume was made up with purified water. 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 and 0.8 ml of this solution was taken in eight 10 ml volumetric flasks and volume was made with clear fresh dog plasma.

(iii) Procedure :

0.2 ml of 6 N hydrochloric acid was added to each flask and aliquots (6 ml) of plasma were transferred to glass stoppered centrifuge tubes. To each centrifuge tube 6 ml of 0.4 N hydrochloric acid, 0.2 ml of ascorbic acid solution and 0.5 ml p-methoxybenzaldehyde solution were added. The mixture was incubated for 30 minutes at 70°C. The tubes were cooled to room temperature and the pH was adjusted to 9.0 with 5 N sodium hydroxide solution. The resulting hydrazone derivative was extracted by shaking each tube on a mechanical shaker for 20 minutes with 30 ml of benzene-methanol mixture. The organic phase was separated by centrifugation, and 25 ml aliquot was transferred to another centrifuge tube. The solvent was evaporated to dryness. The residue was dissolved in 3 ml of n-heptane and mixed with 3 ml of 0.2 N hydrochloric

acid solution. The tubes were shaken in mechanical shaker for 20 minutes. Aqueous phase was separated by centrifugation and absorbance of aqueous layer was measured at 355 nm against 0.2 N hydrochloric acid as blank. Observations are recorded in Table 2-4 and standard calibration curve is plotted in Fig. 2-4.

III. ESTIMATION OF DRUGS IN PRESENCE OF COATING MATERIALS AND EXCIPIENTS

A. TETRACYCLINE HYDROCHLORIDE

The coating materials and excipients were weighed and mixed/dissolved in tetracycline hydrochloride solution in 0.1 N hydrochloric acid. Percent transmission of these solutions was measured at 355 nm using 0.1 N hydrochloric acid as blank. Observations are recorded in Table 2-5.

B. HYDRALAZINE HYDROCHLORIDE

The coating materials and excipients were weighed and mixed/dissolved in hydralazine hydrochloride solution. Percentage transmission was noted at 510 nm after necessary treatment as described earlier. Observations are recorded in Table 2-6.

TABLE 2-3 : ESTIMATION OF HYDRALAZINE HYDROCHLORIDE IN DISSOLUTION FLUID

S.No.	Concentration (µg/ml)	Absorbance
1	50	0.09
2	100	0.19
3	150	0.28
4	200	0.37
5	250	0.46
6	300	0.55
7	350	0.64
8	400	0.83

TABLE 2-4 : ESTIMATION OF HYDRALAZINE HYDROCHLORIDE IN DOG PLASMA

S.No.	Concentration (µg/ml)	Absorbance
1	0.2	0.035
2	0.4	0.075
3	0.6	0.110
4	0.8	0.145
5	1.0	0.180
6	1.2	0.220
7	1.4	0.255
8	1.6	0.280

FIG.·2·3: CALIBRATION CURVE OF HYDRALAZINE HYDROCHLORIDE IN DISSOLUTION MEDIUM.

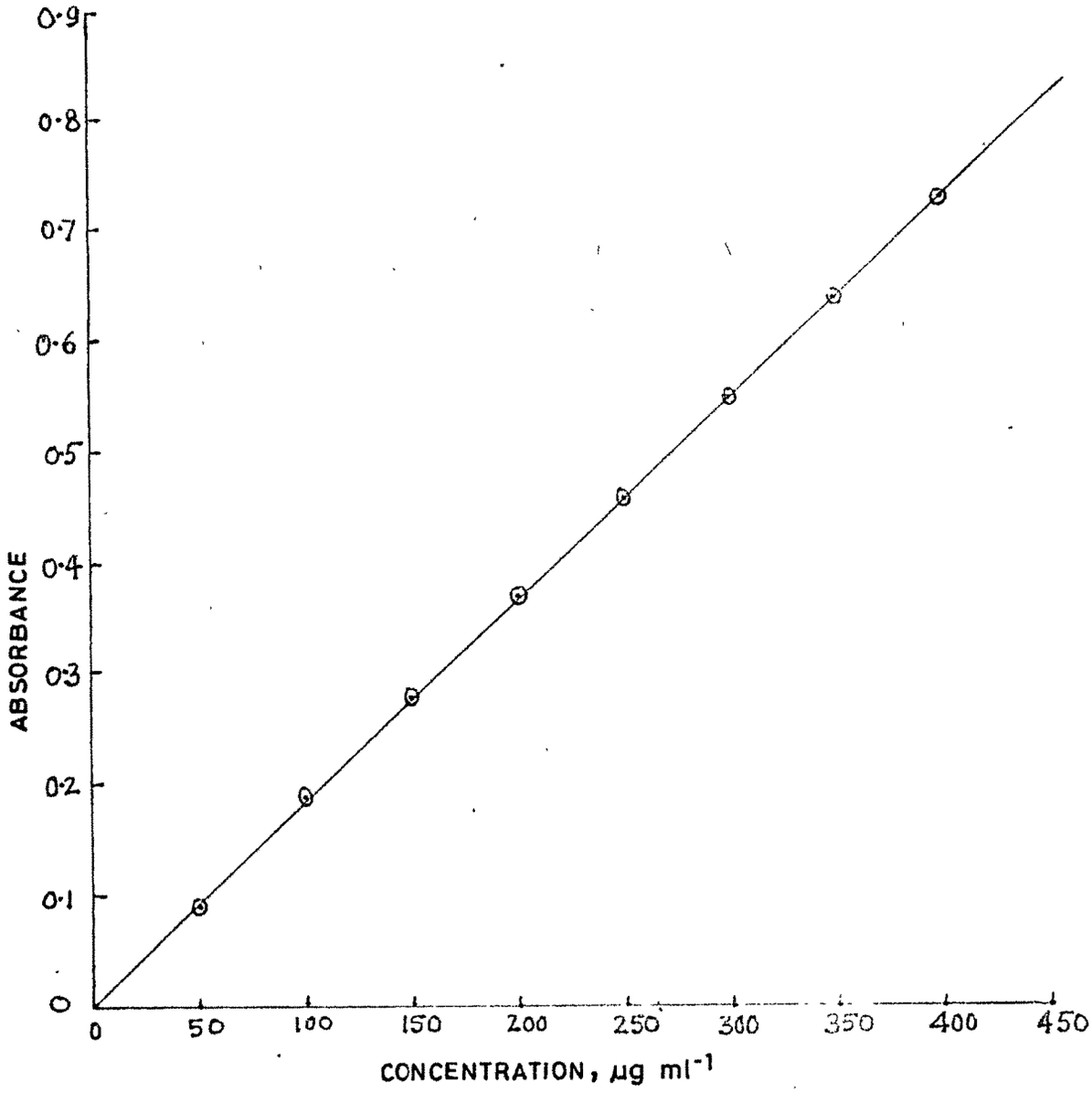
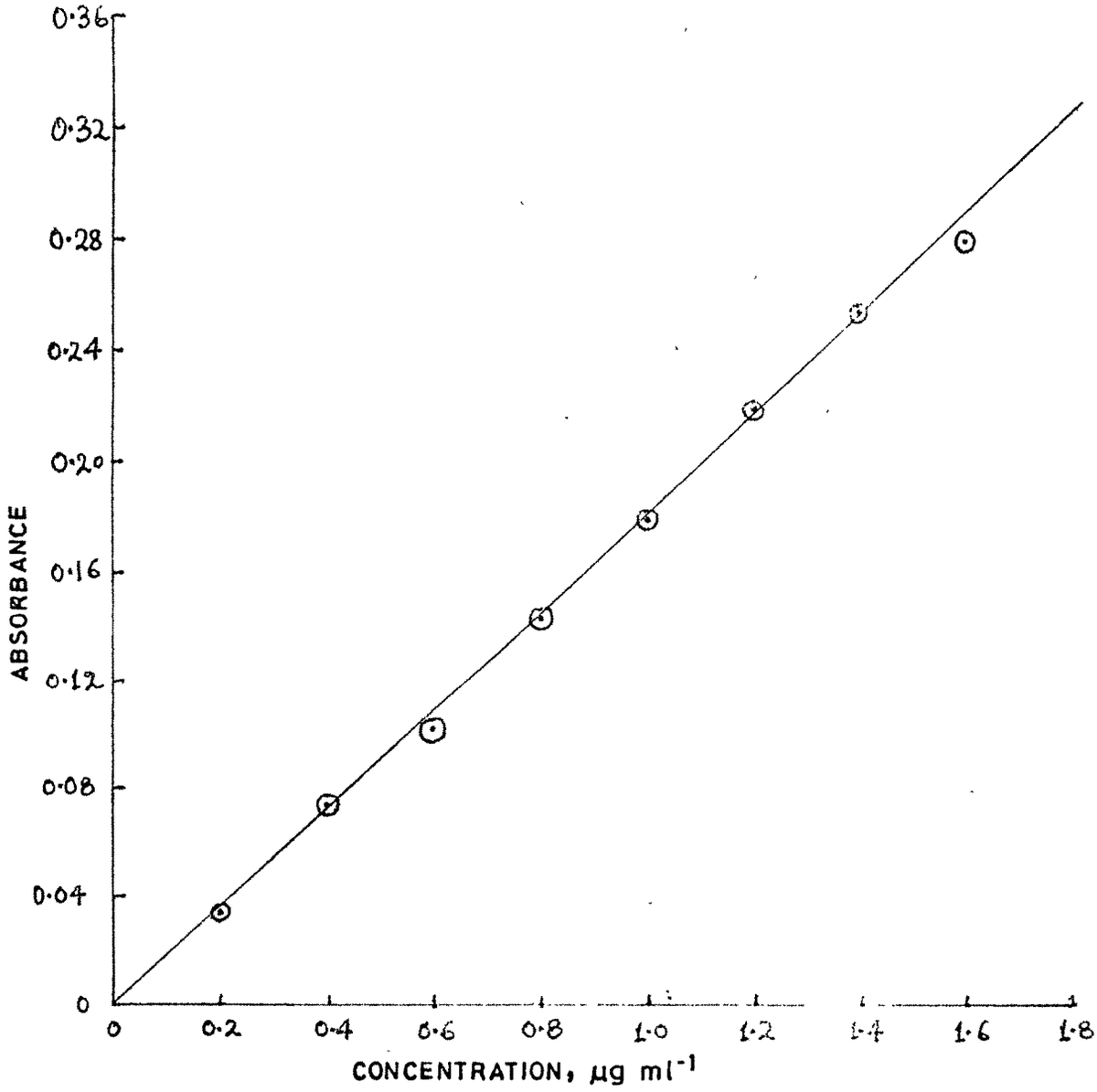


FIG.- 2-4: CALIBRATION CURVE OF HYDRALAZINE HYDROCHLORIDE IN DOG PLASMA.



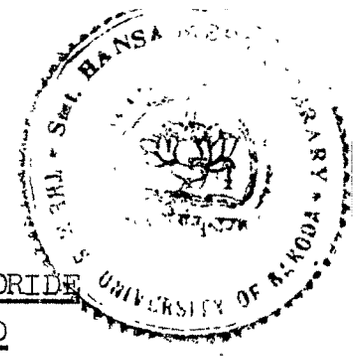


TABLE 2-5 : ESTIMATION OF TETRACYCLINE HYDROCHLORIDE IN PRESENCE OF COATING MATERIALS AND EXCIPIENTS

S.No.	Ingredients	Percentage Transmission	Interference
1.	-	65.0	Nil
2.	Sodium chloride	65.0	"
3.	Potassium phosphate monobasic	65.0	"
4.	Polyvinyl pyrrolidone	64.5	"
5.	Ethyl cellulose	65.5	"
6.	Glyceryl monostearate	64.0	"
7.	Glyceryl distearate	65.0	"
8.	White Bees wax	65.0	"
9.	Shellac	65.0	"
10.	Cellulose acetate phthalate	64.5	"
11.	Eudragit S	65.0	"
12.	Eudragit RL	65.0	"
13.	Eudragit RS	65.0	"
14.	Succinic acid	64.5	"
15.	Silica gel	65.0	"
16.	Avicel	65.0	"
17.	Lactose	64.5	"
18.	Tween-80	66.5	"
19.	Propylene glycol	66.0	"
20.	Dibutyl phthalate	65.0	"
21.	Triacetin	65.0	"
22.	Magnesium stearate	66.0	"
23.	Talc	66.0	"
24.	Sodium hydroxide	65.5	"

TABLE 2-6 : ESTIMATION OF HYDRALAZINE HYDROCHLORIDE IN PRESENCE OF COATING MATERIALS AND EXCIPIENTS.

S.No.	Ingredients	Percentage Transmission	Interference
1.	-	63.0	Nil
2.	Sodium chloride	63.0	"
3.	Sodium phosphate monobasic	63.0	"
4.	Sodium hydroxide	62.5	"
5.	Glyceryl monostearate	64.0	"
6.	Glyceryl distearate	63.5	"
7.	White Bees wax	63.0	"
8.	Eudragit S	63.5	"
9.	Eudragit RL	63.0	"
10.	Eudragit RS	63.0	"
11.	Avicel	62.0	"
12.	Lactose	64.5	"
13.	Tween-80	64.0	"
14.	Propylene glycol	63.0	"
15.	Dibutyl phthalate	63.5	"
16.	Triacetin	63.5	"
17.	Magnesium stearate	64.0	"

RESULTS AND DISCUSSION

The calibration curves prepared for tetracycline hydrochloride and hydralazine hydrochloride are linear plots and both the drugs can be estimated quantitatively by selected spectrophotometric methods within the concentration range (tetracycline hydrochloride 5-40 µg/ml; hydralazine hydrochloride 50-400 µg/ml). Methods reported by Mahgoub *et al.*, and Hall were utilized to develop the method of estimation of tetracycline hydrochloride in human urine for present investigation. The method is based on formation of yellow complex, uranyl-tetracycline with an absorption maximum at 430 nm. It follows Beer's law at concentration range of 2.5 to 35 µg/ml. The method reported by Zak *et al.* was used for estimation of hydralazine hydrochloride in plasma. Spectrophotometric technique for estimation of hydralazine is based on reaction with an absorption maximum at 355 nm. It obeys Beer's law in concentration range of 0.2 to 1.6 µg/ml.

Estimation of drugs in presence of coating materials and excipients revealed that materials tested do not interfere in the estimation.

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