CHAPTER 2 : *Spectrophotometric Analysis*

CHAPTER 2

SPECTROPHOTOMETRIC ANALYSIS.

This chapter deals with the details regarding instrumentation, reagent preparation, procedure for colour development, linearity study, validation of methods, and also results and discussion of all the spectrophotometric methods developed.

2.1 EXPERIMENTAL

Instrumentation: All spectral and absorbance measurements were made on a Shimadzu UV-Vis Spectrophotometer (UV-260 or UV-160) with one cm matched quartz cells. pH measurements were made on an Elico digital pH meter.

Reagents: All the chemicals used were of AR grade. Reagents were supplied by BDH Chemicals, E. Merck, Loba Chemicals, SD Chemicals, Sisco Lab and Qualigens (India) and Fluka Chemie AG (Switzerland). Following reagent solutions were prepared for use in different methods:

1. Ferric chloride (BDH) : A 0.2% w/v solution was prepared in alcohol.

2. 2,2'-Bipyridine (BDH) : A 0.5% w/v solution was prepared in alcohol.

3. Sodium nitroprusside (BDH): A 1% w/v solution was prepared in water.

4. Ion-pair reagents (BDH, Loba, SD): The reagents used in the determination of DFS are 0.05% solutions of acridine orange (AO), basic fuchsin (BF), methylene blue (MB), safranine (SF) and toluidine blue (TB) in distilled water. The reagents used in the determination of KTR are 0.05% solutions of MB and SF. The reagents used in the determination of DLZ are 0.1% solutions of bromocresol green(BCG), bromocresol purple(BCP), bromophenol blue(BPB), bromothymol blue(BTB),

solochrome dark blue(SDB), methyl orange(MTO), eriochrome black T(EBT), tropaeolin OO(TPO) and picric acid in water. Each dye (100 ml) was extracted with 30 ml portions of chloroform to remove soluble impurities.

5. Ammonium persulphate (E.Merck): A 2% (w/v) solution was prepared in water.

6. Dimethylaminobenzaldehyde (E.Merck): A 0.25% (w/v) solution was prepared in methanol.

7. Cetrimide (Loba) : A 2% (w/v) solution was prepared in water.

8. Sodium hypobromite : The solution was prepared by dissolving 1.1 ml of bromine (E.Merck) in 100 ml of 2% (w/v) sodium hydroxide solution and further diluting 6.6 ml of this solution to 100 ml with the same solvent.

9. Sodium nitrite (BDH) : A 5% (w/v) solution was prepared in distilled water.

10. Cobalt thiocyanate reagent : The reagent was prepared by dissolving 20.4 g of cobalt chloride (E.Merck) and 12.9 g of ammonium thiocyanate (E.Merck) in 40 ml water.

11. Iron (III) chloride (for DLZ determination): A 1.0 M iron (III) chloride solution was prepared in 0.1 M hydrochloric acid.

12. Ammonium thiocyanate (for DLZ determination): A 4.0 M solution was prepared in water.

13. N-Bromosuccinimide (Loba): A 0.05 M solution of NBS was freshly prepared in methanol.

14. Dibromo dimethyl hydantoin (Fluka): A 0.025 M solution of DDH was freshly prepared in methanol.

15. Ceric ammonium sulphate (E. Merck): A 0.1 M solution was prepared by dissolving 6.6 g of the reagent in a mixture of 3 ml sulphuric acid and 40 ml water and further diluting it to 100 ml with water.

16. Potassium bromate (BDH): A 0.12 M solution was prepared in water.

17. Hydroxylamine reagent : An aqueous solution was prepared by mixing equal volumes of 12.5% (w/v) hydroxylamine hydrochloride (BDH) and 12.5% (w/v) of sodium hydroxide (BDH).

18. Ferric reagent (for DLZ) : 1.25 g of ferric ammonium sulphate (SD) was dissolved in 10 ml of 70% (w/w) perchloric acid solution and diluted to 100 ml with water.

19. Dilute perchloric acid solution : A 14% (w/v) solution was prepared by diluting 10 g of 70% (w/w) perchloric acid solution to 50 ml with water.

20. DDQ solution (Sisco) : A 0.2% (w/v) solution was prepared in methanol.

21. DCNP (Fluka) solution : A 0.2% (w/v) solution was prepared in methanol.

22. Iodine (BDH) solution : A 0.05% (w/v) solution was prepared in chloroform.

23. Chloranil (Fluka) solution : A 0.2% (w/v) solution was prepared by dissolving 100 mg of the reagent in 20 ml of toluene and 80 ml of methanol.

The following buffer solutions were prepared :

1. Mixed phosphate buffers (0.1 M, pH 6.8, 7.0 and 7.4): To 500 ml of 0.1 M potassium dihydrogen orthophosphate a sufficient quantity of 0.2 M disodium orthophosphate was added to get the required pH and the solution was diluted to 1000 ml with water.

2. Phthalate buffers. (pH 1.5, 2.5, 4.5, 4.7 and 5.0): The buffer solution of required pH was prepared by mixing 0.05 M solution of potassium hydrogen phthalate with appropriate quantity of dilute hydrochloric acid or dilute sodium hydroxide.

3. Ammonia buffer (pH 10): This was prepared by dissolving 1.69 g of ammonium chloride (BDH) and 14.3 ml of ammonia solution in one liter of water.

4. Acetate buffer of pH 4 : Prepared by mixing 10.8 g of sodium acetate (BDH) and 57 ml of acetic acid, further diluting it to 200 ml with water.

The other reagents used in the study are:

1. Distilled Chloroform (E. Merck), Methanol (Qualigens), Benzene (E. Merck) and Toluene (E. Merck).

2. Hydrochloric acid solution: Solutions of different concentration viz 0.1 M, 0.2 M and 1.0 M were prepared by appropriate dilution of concentrated acid with water.

3. Sulphuric acid solution: Solutions of different concentration viz 0.05 and 0.1M were prepared by appropriate dilution of concentrated acid with water.

4. Sodium hydroxide solution: Solutions of different concentrations viz 0.1 M, 0.2 M

__ and 1.0 M were prepared by appropriate dilution of the stock solution of the reagent in water.

PREPARATION OF STANDARD STOCK SOLUTION

Diclofenac sodium reference standard was obtained from M/s Quantum Chemicals Co. Ltd (Taiwan). Diltiazem hydrochloride and desacetyl diltiazem hydrochloride were obtained from M/s Orion Co. Ltd (Finland). Famotidine was obtained from M/s Cheminor Drugs Ltd (India). Ketorolac tromethamine was obtained from M/s Lupin Labs Ltd. (India).

Diclofenac sodium: Stock solutions (1.0 $mg.ml^{-1}$ and 0.5 $mg.ml^{-1}$)of DFS was prepared by dissolving 100 mg and 50 mg of the drug separately in water and diluting to 100 ml with water. These solutions were used in methods A-1, A-5, A-6 and A-7. Further dilutions of stock solution were made with buffers of pH 6.8, 7.0 and 7.4 separately to get a concentration of 60 $\mu g.ml^{-1}$. These solutions were used in methods A-8, A-9, A-10, A-11 and A-12.

Stock solutions of DFS were prepared separately in methanol containing 0.4 mg, 1.0 mg, 2.0 mg of the drug per ml. These solutions were used in methods A-2, A-3, A-4, A-13 and A-14. For the method using iodine (A-15), a stock solution containing 1 mg of drug per ml was prepared by dissolving 25 mg of DFS in 1 ml of methanol before diluting to 25 ml with chloroform.

Diltiazem hydrochloride: Stock solutions of 0.25 mg, 1 mg and 3 mg per ml were prepared by dissolving separately 25 mg, 100 mg and 300 mg of DLZ in 100 ml water. These solutions were used in methods B-3, B-4, B-5, B-6, B-7, B-8, B-9, B-10, B-11, B-12 and B-13. Stock solutions of 1 $mg.ml^{-1}$ and 4 $mg.ml^{-1}$ were prepared by dissolving 25 mg and 100 mg of DLZ in 25 ml of methanol. These

solutions were used in methods B-1, B-2 and B-14.

Famotidine: Stock solutions of 0.5 $mg.ml^{-1}$ and 1 $mg.ml^{-1}$ were prepared by dissolving 50 mg and 100 mg of FMD in 100 ml methanol. These solutions were used in methods C-1, C-2, C-5, C-6, C-7 and C-8. A solution containing 1 $mg.ml^{-1}$ was prepared by dissolving 50 mg of FMD in 50 ml of 1 N HCl and used in method C-3. A solution containing 1 $mg.ml^{-1}$ was prepared by dissolving 50 mg of FMD in 50 ml of 0.1 N HCl and used in method C-4.

Ketorolac tromethamine: A 200 $\mu g.ml^{-1}$ stock solution of KTR in methanol was used in methods D-3 and D-4. A 250 $\mu g.ml^{-1}$ stock solution of KTR in water was used in ion-pair complexation methods D-1 and D-2.

2.2 METHODS BASED ON OXIDATION REACTIONS

DFS, DLZ and FMD have been analysed by methods based on oxidation reactions using the reagents, viz N-bromosuccinimide and dibromo dimethyl hydantoin. DFS was also analysed by using oxidative reagents like ceric ammonium sulphate and potassium bromate. FMD was reacted with sodium nitrite in acidic medium in which the nitroso compound produced developed a yellow colour on treatment with alkali, which was measured spectrophotometrically at 440 nm.

2.2.1 PROCEDURES:

Method A-1 for DFS: Aliquots of aqueous solution of DFS (1-5 ml, 0.1 $mg.ml^{-1}$) were transferred into a series of separating funnels. The total volume was adjusted to 5 ml in all the funnels using water and one ml of CAS solution was added. After one min 5 ml of acetate buffer (pH 4.0) and 25 ml of benzene were added. The separating funnels were shaken for 2 min and allowed to stand for the

clear separation of organic layer. The yellow colour of the benzene layer was measured at 450 nm against a reagent blank.

Methods A-2 and A-3 for DFS: Aliquots of methanolic solution of DFS (1-5 ml, 1 $mg.ml^{-1}$) were pipetted out into a series of 10 ml volumetric flasks. To each flask 2 ml of either dibromo dimethyl hydantoin or N-bromosuccinimide solution was added. The volume was made upto 10 ml with methanol and allowed to stand at 23-25°C for 15 min. The absorbance was measured at 385 nm against a reagent blank.

Method A-4 for DFS: Aliquots of aqueous solution of DFS (0.5 - 3.0 ml, 2 $mg.ml^{-1}$) were pipetted out into a series of 10 ml volumetric flasks. The volume was adjusted to 3 ml in all the flasks using methanol. To each flask one ml of potassium bromate solution and one ml of 0.05 M sulphuric acid solutions were added. The flasks were kept at 73-75°C for 5 min. After cooling, the volume was made upto 10 ml with methanol. Absrobance was measured at 495 nm against a reagent blank.

Methods B-1 and B-2 for DLZ: Aliquots of methanolic solution of diltiazem hydrochloride (1 $mg.ml^{-1}$, 1-5 ml) were pipetted out into a series of 10 ml volumetric flasks followed by 3 ml of DBH or NBS solution. The volume was made upto 10 ml with methanol and allowed to stand at 25°C for 30 min. The absorbance was measured at 386 nm against a reagent blank.

Methods C-1 and C-2 for FMD: Aliquots of methanolic solution of FMD (0.5 $mg.ml^{-1}$, 1-5 ml) were treated either with 3 ml of DBH or NBS solution in 10 ml volumetric flasks. The volume was made upto 10 ml with methanol. The absorbance was measured at 386 nm after 10 min against a reagent blank.

For tablet analysis, tablet powder equivalent to 20 mg of FMD was shaken with 100 ml methanol. Five ml of the filtrate was used for the colour development procedure described as above.

Method C-3 for FMD: Aliqouts of standard solution in 1 N HCl (1 $mg.ml^{-1}$, 1-5 ml) were pipetted out into a series of 10 ml volumetric flasks. The volume was adjusted to 5 ml in all the flasks using 1 N HCl. Into each flask 1.5 ml of sodium nitrite solution was added. The solution was allowed to stand for 15 min at 15°C. Then 3.5 ml of 2 N sodium hydroxide were added and the contents were mixed. After 30 min, the absorbance was measured at 440 nm against a reagent blank.

Tablet powder equivalent to 20 mg of the drug was shaken with 20 ml of 1 N HCl and diluted to 50 ml with the same solvent. 5ml of filtrate was used for colour development by the method described above.

2.2.2 RESULTS AND DISCUSSION

Methods A-1 to A-4 for DFS: Diclofenac sodium was found to yield intense coloured products with CAS, DBH, NBS and PBT in aqueous or methanolic medium (Fig. 2.1) probably due to oxidation of the drug as these reagents are known oxidising agents[3]. The relative sensitivity of the methods based on these reactions for the estimation of the drug can be compared by apparent molar absorptivity values of chromogens (Table 2.1).

Factors affecting the reaction viz pH, reaction medium, heating time and reagent concentration were carefully studied to achieve quantitative results.

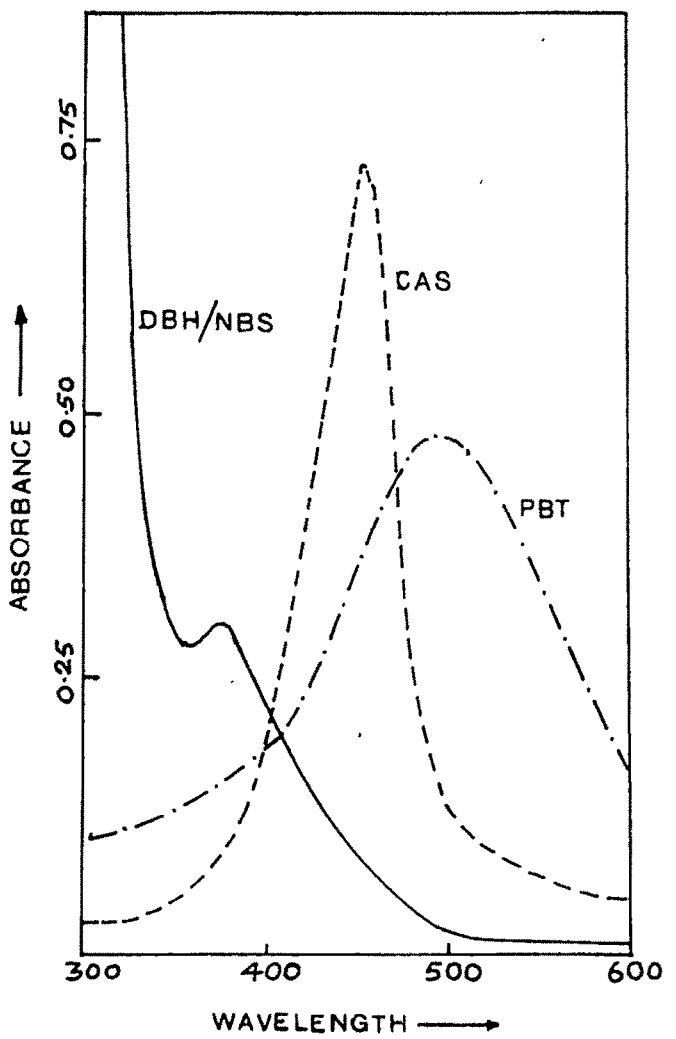


Fig 2.1a : Absorption Spectra of Oxidation Product of DFS with DBH (———), NBS (———), CAS (- - - -) and PBT (—·—·—)

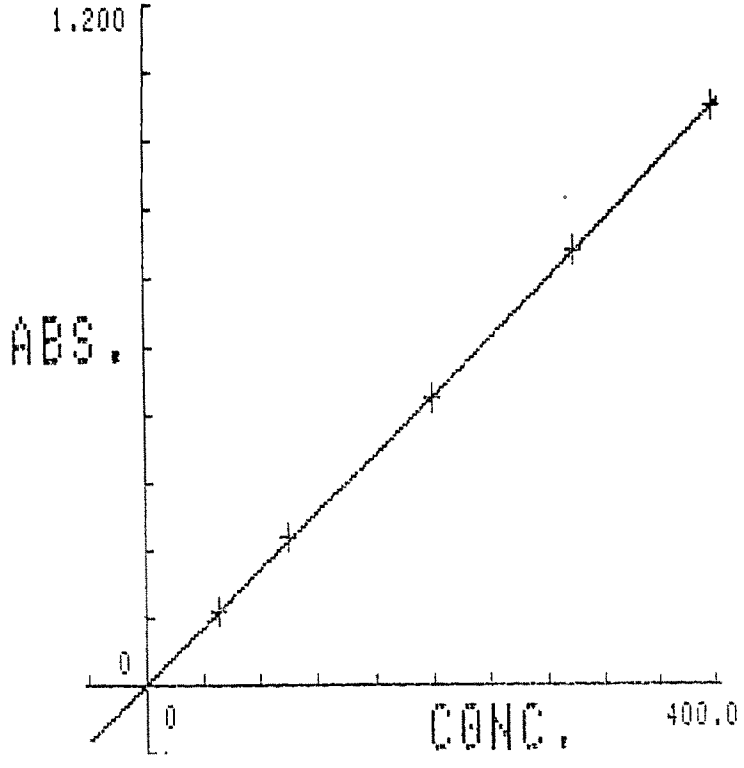


Fig. 2.1b : Linearity plot of DFS-DBH method

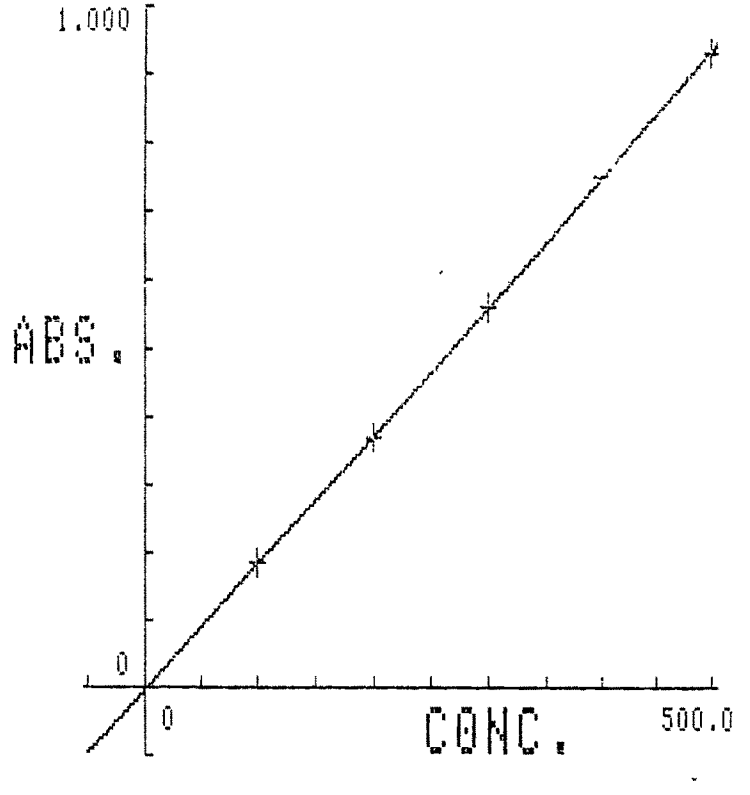


Fig. 2.1c : Linearity plot of DFS-NBS method

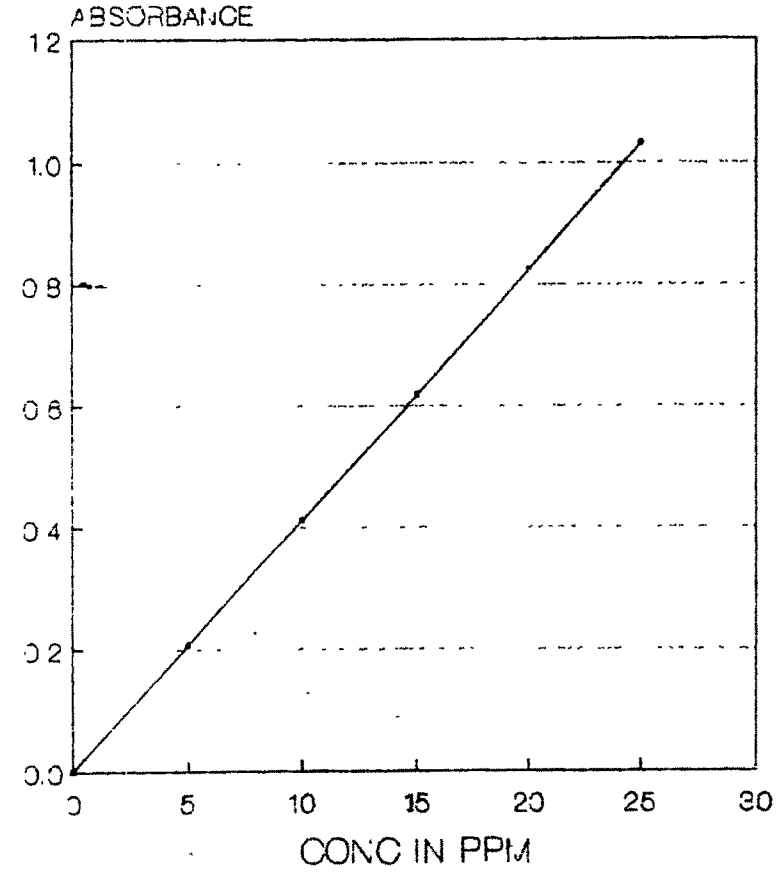


Fig. 2.1d : Linearity plot of DFS-CAS method

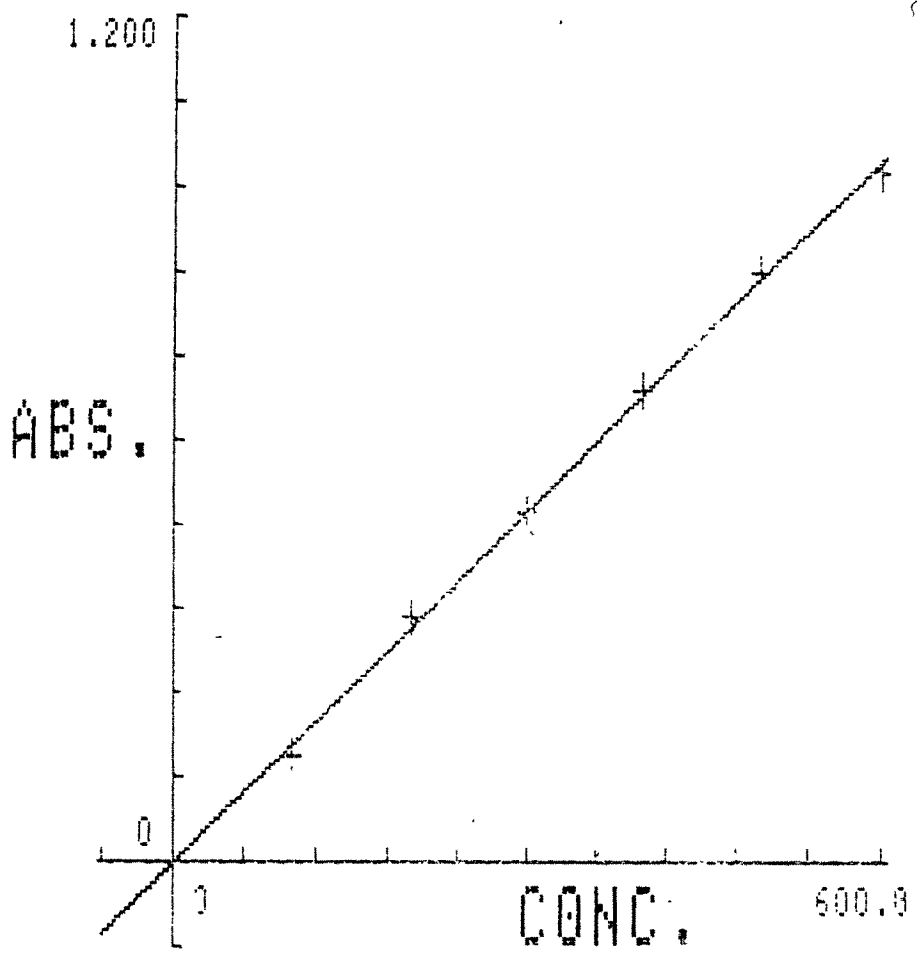


Fig. 2.1e : Linearity plot of DFS-PBT method

Table 2.1 : Spectral data for the reaction products of DFS with CAS, DBH, NBS and PBT.

Reagent	λmax (nm)	Linearity range $\mu g.ml^{-1}$	Molar absorptivity $L.mol^{-1}.cm^{-1}$	Intercept	correlation coefficient
CAS	450	2-16	$2.108x10^4$	0.0219	0.9994
DBH	385	40-400	$0.834x10^3$	0.0121	0.9999
NBS	385	50-500	$0.538x10^3$	0.0109	0.9999
PBT	495	50-600	$0\ 474x10^3$	0.0389	0.9992

In the case of CAS method extraction with benzene was found to be most suitable. The order of addition that gave optimal results was: CAS reagent followed by the buffer and then benzene. The colour was measured within 10 min, thereafter gradual fading was observed.

In the methods using DBH and NBS, methanolic medium showed higher sensitivity as compared to aqueous medium. It was established that 2 ml of either reagent was sufficient to give maximum colour in the range of the drug concentration studied. Maximum colour was observed after 15 min.

In PBT method, the total volume of the aqueous components of the reaction mixture was optimised to 2 ml which prevented the precipitation of the reaction products but gave optimal colour.

The practicality and suitability of the proposed methods for quantitation of DFS can be judged by the results of tablet and injectable analysis given in Table 2.2.

Table 2.2 : Results of diclofenac tablets and injectable analyses by the method using CAS, DBH, NBS, PBT.

Formulation	Label claim(mg)	% Found (± SD)			
		CAS	DBH	NBS	PBT
Tablet*	50 per tablet	98.17 (±0.65)	98.22 (±0.52)	98.24 (±0.48)	98.18 (±0.46)
Injectable*	25 per ml	99.62 (±0.72)	99.59 (±0.64)	99.57 (±0.58)	99.85 (±0.54)

* Products of CIBA GIEGY, GERMANY.

For recovery studies known amount of the pure drug for three different levels were added to measured aliqouts of the tablet and injectable solutions. The solutions were reanalysed and the amount of the drug was calculated from the calibration graph. The percent recovery was found by using the formula

$$\% \text{ Recovery} = \frac{N(\Sigma XY) - (\Sigma X)(\Sigma Y) \times 100}{N(\Sigma X^2) - (\Sigma X)^2}$$

where X = amount of standard drug in μg

Y = amount of drug found in μg

N = total amount of observations

The recovery of the drug was in the range of 97.3 to 100.2%. This showed the absence of interference from common excipients used in tablet and injectable formulations. However, interference was observed in the presence of reducing agents. In such cases DFS was first extracted from an acidic solution with ether. After evaporation of ether the residue was redissolved in methanol or in minimum quantity of dilute alkali. The methods gave concordant results with good reproducibility. Paracetamol, if present, was found to interfere and therefore, the methods can not be applied in such cases.

Methods B-1 and B-2 for DLZ: Diltiazem hydrochloride was found to yield yellow coloured chromogen with reagent DBH or NBS most probably due to oxidation. Fig 2.2 shows the absorption spectra of coloured products formed with wavelength of maximum absorption at 386 nm. Spectral data for the reaction products of DLZ with both the reagents are given in Table 2.3.

Table 2.3 : Spectral data for reaction products of DLZ with DBH and NBS

Reagent	λmax (nm)	Linearity range µg/ml	Molar absorptivity $L.mol^{-1}.cm^{-1}$	Intercept	Correlation coefficient
NBS	386	40-450	$0.943x10^3$	0.0101	0.9998
DBH	386	30-320	$1.429x10^3$	0. 0365	0.9999

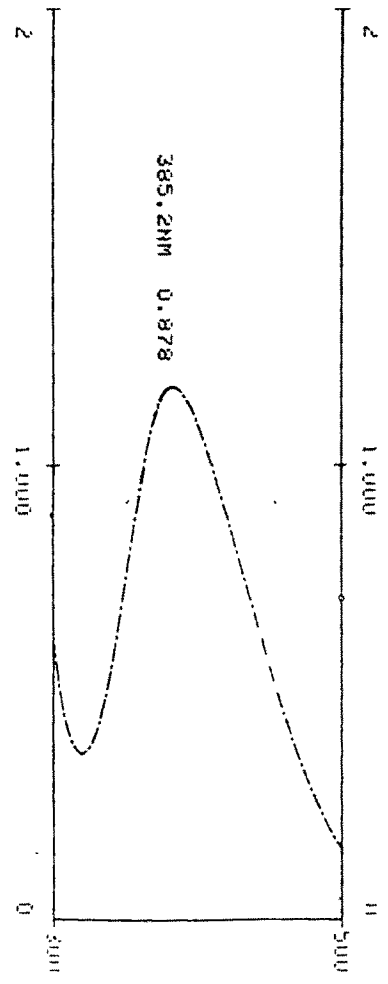


Fig. 2.2a : Absorption spectra of oxidation product of DLZ with NBS

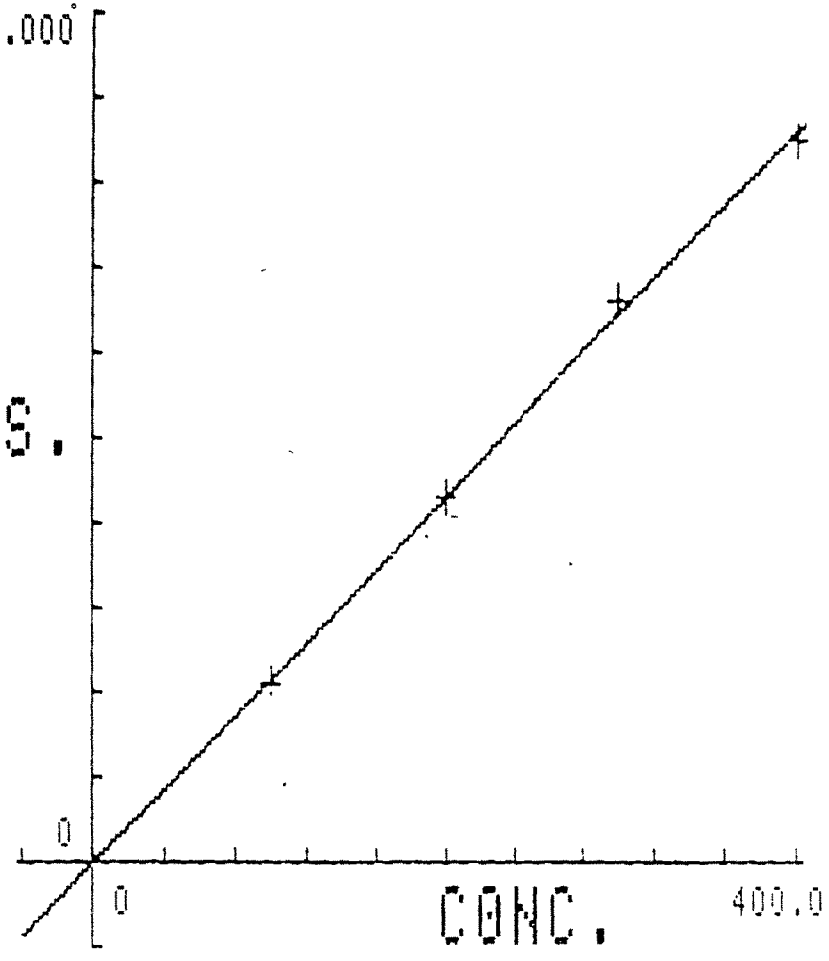


Fig. 2.2b : Linearity plot of DLZ-NBS method

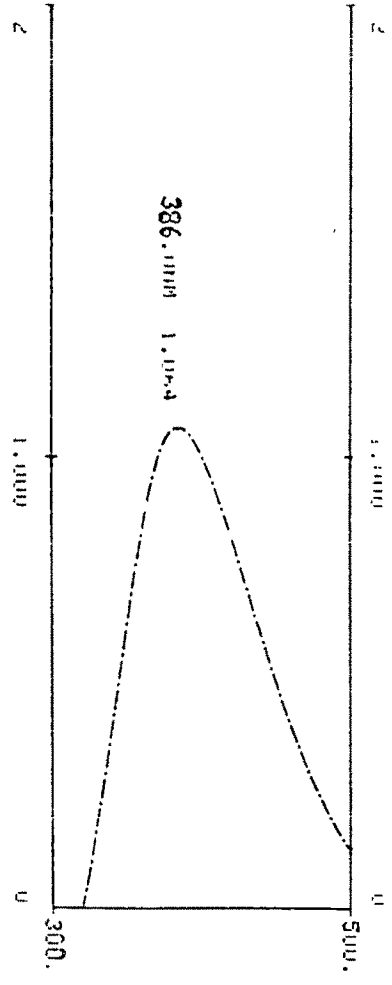


Fig. 2.2c : Absorption spectra of oxidation product of DLZ with DBH

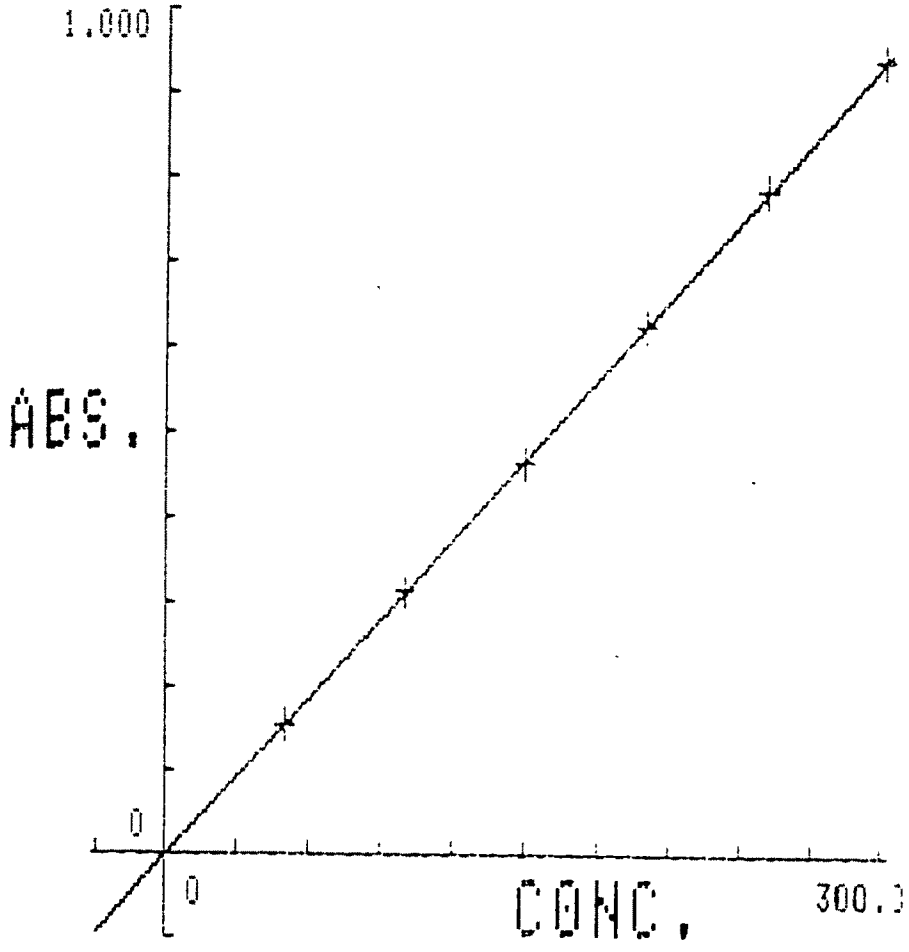


Fig. 2.2d : Linearity plot of DLZ-DBH method

Several experiments were conducted to fix the optimum parameters like concentration of the reagent, reaction time and solvent for the reaction. It was found that at lower concentration of the oxidising reagent, a yellow colour develops which quickly fades. An optimum of 3 ml of DBH or NBS reagent was required for stable and maximum colour. The time required for maximum colour development was 30 min and the colour remained stable for 3 hours. Among the various solvents used as the reaction medium, methanol gave satisfactory and more stable colour.

Marketed products of diltiazem tablets were analysed by the proposed methods and the results are given in Table 2.4.

Table 2.4: Results of diltiazem analysis by DBH and NBS method.

Tablet	Label claim mg	% Found (± SD)		
		NBS method	DBH method	USP method
Lot A	30	97.28(±0.86)	97.30(±0.45)	97.25(±0.38)
Lot B	30	98.12(±0.54)	98.16(±0.41)	98.13(±0.42)
Lot C	60	98.45(±0.72)	98.38(±0.56)	98.36(±0.26)
Lot D	60	99.52(±0.64)	99.49(±0.58)	99.43(±0.28)

The recovery of the drug added to the preanalysed sample solution were found in the range of 98.65-100.48% which showed the absence of interference from common excipients used in tablet formulations. The results obtained by the proposed methods are comparable with those of the USP method[10].

Methods of C-1 and C-2 for FMD: Famotidine was found to yield intense yellow colour with reagents DBH and NBS due to oxidation of the drug by these reagents. The relative sensitivity of both the reagents in the estimation of the drug can be seen from the apparent absorptivity values of chromogens (Table 2.5). Absorption spectra of the reaction products are given in Fig. 2.3.

Table 2.5: Results of famotidine tablet analysis by DBH and NBS.

Reagent	Linearity range µg/ml	Molar absorp-tivity $L.mol^{-1}.cm^{-1}$	Amount found by proposed method mg/tab*	SD %	% claim
DBH	12-125	$2.820x10^3$	19.452	±0.86	97.26
NBS	14-125	$2.496x10^3$	19.628	±0.75	98.14

* Labelled amount : 20 mg/tablet

Several experiments were conducted to fix the parameters, viz concentration of the reagent, reaction time and solvent for the reaction. It was established that 3 ml of the reagents were required for maximum colour development. The time required for maximum colour development was 10 min. The practicality and suitability of the proposed methods can be judged by the results of tablet analysis given in Table 2.5.

For recovery studies known amount of the drug was added to the tablet solution which had been analysed earlier. The recovery of the drug was in the range of 98.46-100.60% which showed the absence of interference from common excipients used in tablet formulations.

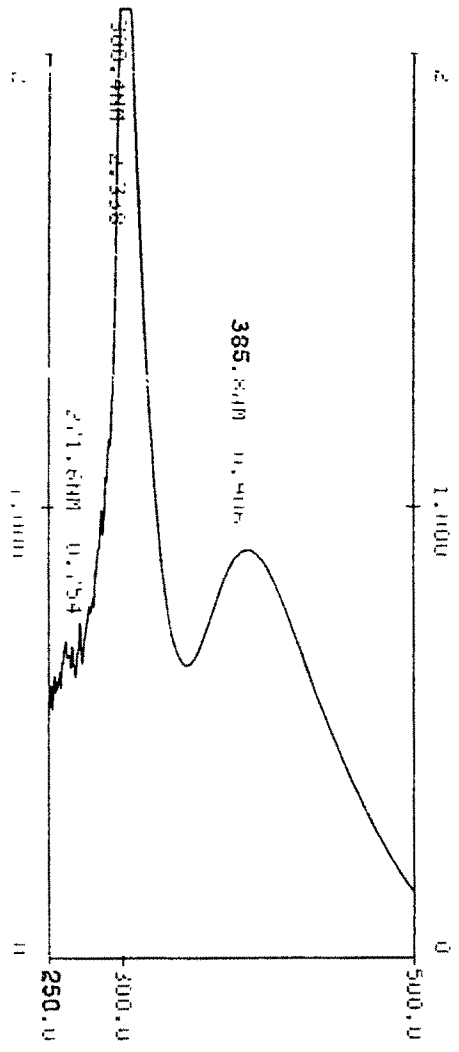


Fig. 2.3a : Absorption spectra of oxidation product of FMD with NBS

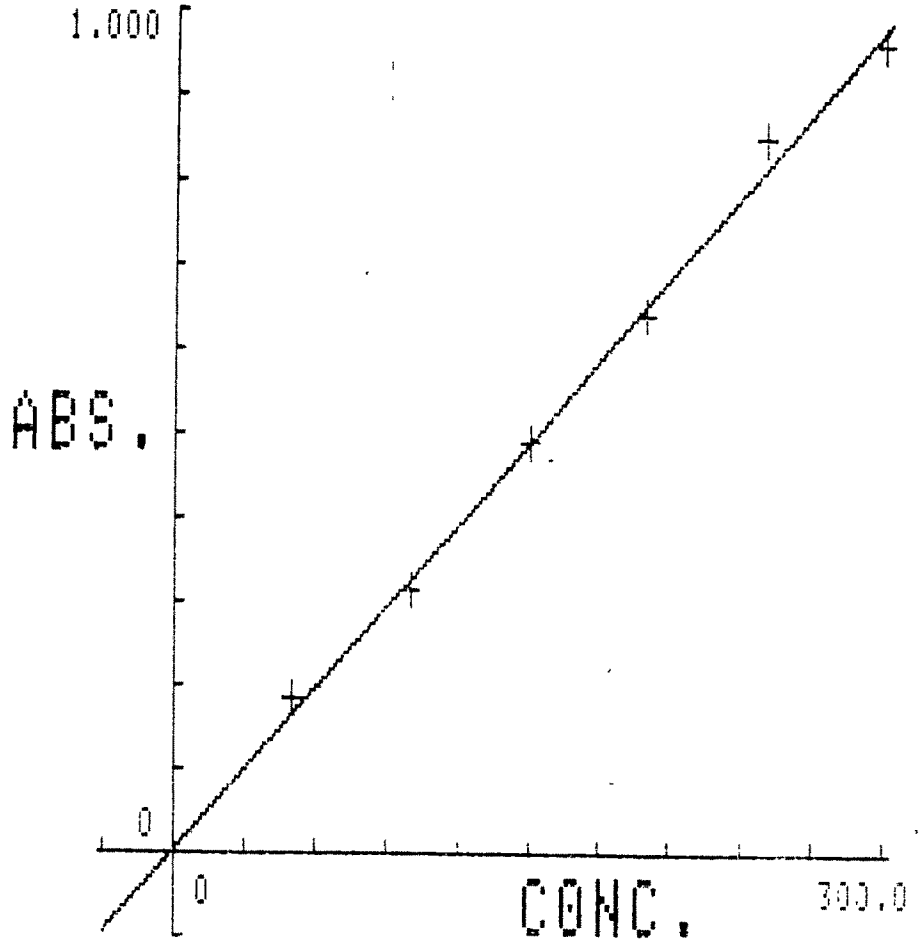


Fig. 2.3b : Linearity plot of FMD-NBS method

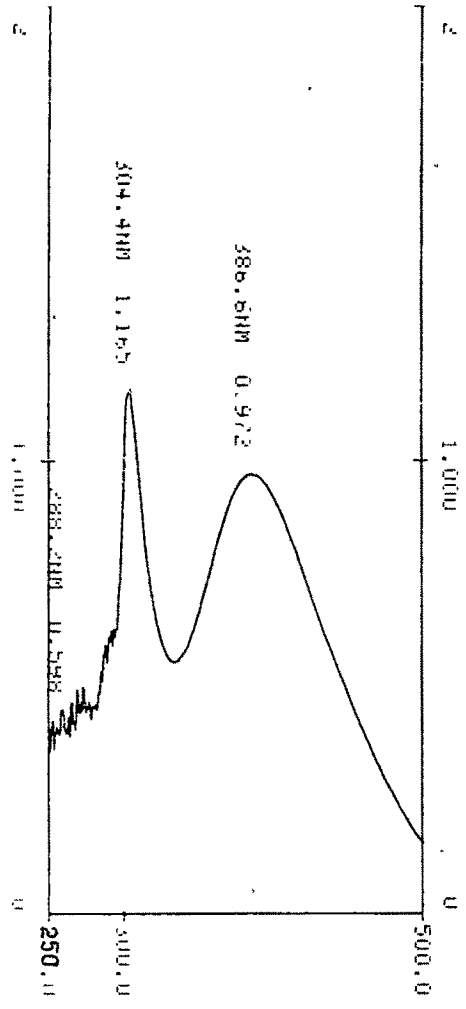


Fig. 2.3c : Absorption spectra of oxidation product of FMD with DBH

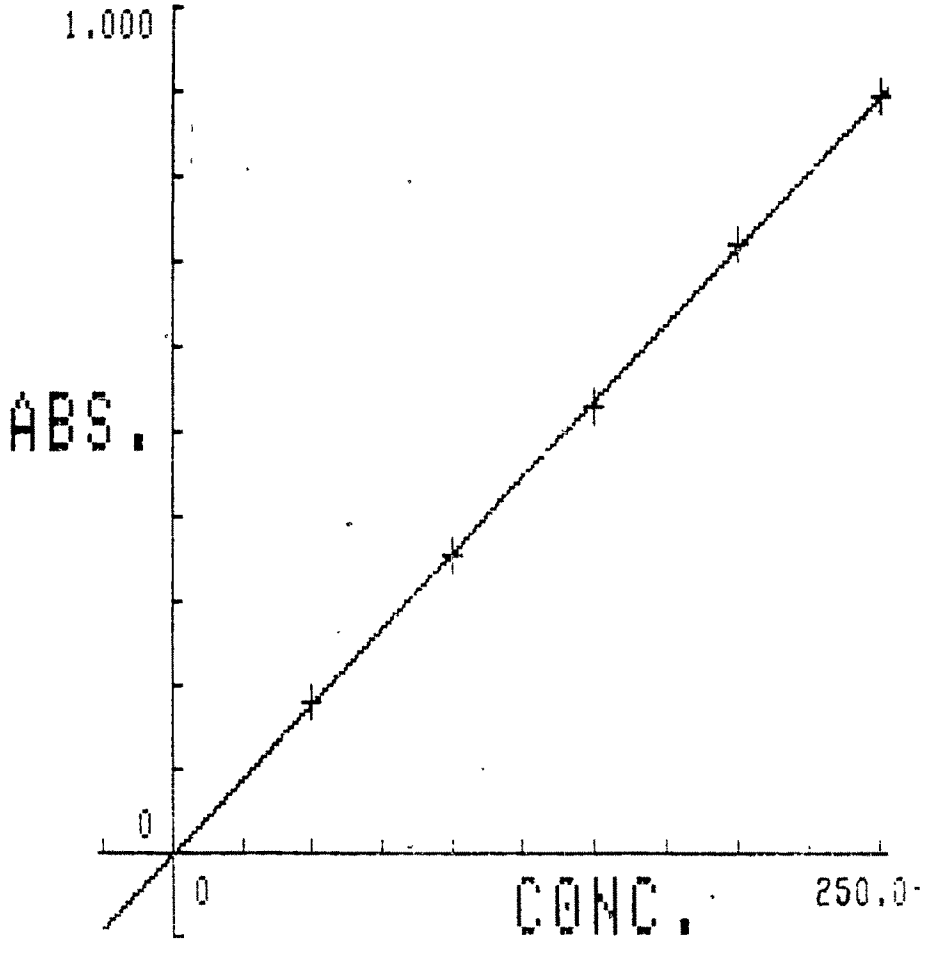


Fig. 2.3d : Linearity plot of FMD-DBH method

Method C-3: Famotidine when treated with sodium nitrite in acidic medium gave a nitroso derivative. On treatment with alkali, the nitroso derivative produced a yellow coloured product with λmax at 440 nm. An optimum time of 15 min was required for diazotization reaction at 15°C. Excess of alkali solution was required to neutralise the initial acidic condition and also to develop the yellow colour. Maximum colour was developed after 30 min on addition of alkali. Spectral characteristics and results of tablet analysis are given in Table 2.6. The colour developed was stable for more than one hour.

Table 2.6: Spectral characteristics and results of FMD tablet analysis by method using sodium nitrite.

λmax (nm)	Linearity range $\mu g.ml^{-1}$	Molar absorptivity $L.mol^{-1}.cm^{-1}$	Amount found mg/tab*	SD %	% claim
400	50-500	0.666×10^3	19.730	0.88	98.65
			39.128	0.75	97.82

* Labelled amount: 20 mg and 40 mg per tablet respectively.

2.3 METHODS BASED ON OXIDATIVE COUPLING REACTIONS

DFS was oxidised with ammonium persulphate in alkaline medium and coupled with dimethylaminobenzaldehyde reagent. The yellow colour produced showed maximum absorbance at 450 nm and linearity in the range 2-12 $\mu g.ml^{-1}$. In another method DFS was treated with sodium hypobromite in presence of cetrimide in alkaline medium producing a yellow colour which was measured spectrophotometrically at 460nm.

2.3.1 PROCEDURES

Method A-5 for DFS: A standard solution containing 20 $\mu g.ml^{-1}$ of DFS was prepared in distilled water. Into a series of 10 ml volumetric flasks aliquots of the drug solution in the range 1-5 ml were pipetted out. Volume was adjusted to 5 ml in all the flasks with water. To each flask one ml ammonia buffer (pH 10) and one ml of ammonium persulphate solution were added. The contents were mixed and allowed to stand for 15 min. Then one ml of dimethylaminobenzaldehyde solution was added and the volume was made upto 10 ml with water. Absorbance was measured at 450 nm after 20 min, using a reagent blank.

Tablet powder or injectable preparation equivalent to 50 mg of DFS was shaken with 30 ml of 0.2 N HCl in a separating funnel. Diclofenac acid was extracted with three 30 ml portions of ether. The total ether layer was evaporated in a 250 ml volumetric flask and the residue was dissolved in 5 ml of 1N sodium hydroxide. The volume was made upto the mark with water and 5 ml of filtrate was further diluted to 100 ml. Five ml solution was used for colour development as described above.

Method A-6 for DFS: Aliquots of aqueous drug solution (0.25 $mg.ml^{-1}$, 1-5 ml) were taken in a series of 10 ml volumetric flasks. To each flask one ml of cetrimide solution and 1 ml of sodium hypobromite solutions were added. The volume was made upto 10 ml with water. The contents were mixed and kept at 18-20°C for 25 min. Absorbance was measured at 460 nm against a reagent blank.

Drug formulation (Tablets and injectables) equivalent to 50 mg of the drug was shaken with 30 ml of 0.2N HCl in a separating funnel. The drug was extracted with three 30 ml portions of ether. The combined ether extract was evaporated in a 200ml

volumetric flask. The residue was dissolved in 5 ml of 1N sodium hydroxide and volume was made upto the mark with water. Five ml of filtrate was used for colour development as described above.

2.3.2 RESULTS AND DISCUSSION

Methods A-5 and A-6 for DFS: The first method utilizes the oxidation of DFS by ammonium persulphate in alkaline medium and coupling with dimethylaminobenzaldehyde reagent. The yellow colour produced shows maximum absorbance at 450 nm and linearity in the range 2-12 $\mu g.ml^{-1}$. In the second method the drug was treated with sodium hypobromite in presence of cetrimide in alkaline medium producing yellow colour which was estimated colorimetrically at 460 nm. Linearity was observed in the range 15-140 $\mu g.ml^{-1}$. Absorption spectra of the resulting solution are shown in Fig. 2.4. Spectral data of both the methods are given in Table 2.7.

Table 2.7 : Spectral data for the reaction products of DFS with DAB and SHB.

Reagent	λmax (nm)	Linearity range µg/ml	Molar absorptivity $L.mol^{-1}.cm^{-1}$	Intercept	Correlation coefficient
DAB	450	2-12	$2.642x10^4$	0.0816	0.9997
SHB	460	15-140	$2.263x10^3$	0.0746	0.9996

Marketed products of diclofenac tablets and injectables were analysed by the proposed methods and the results are summarized in Table 2.8.

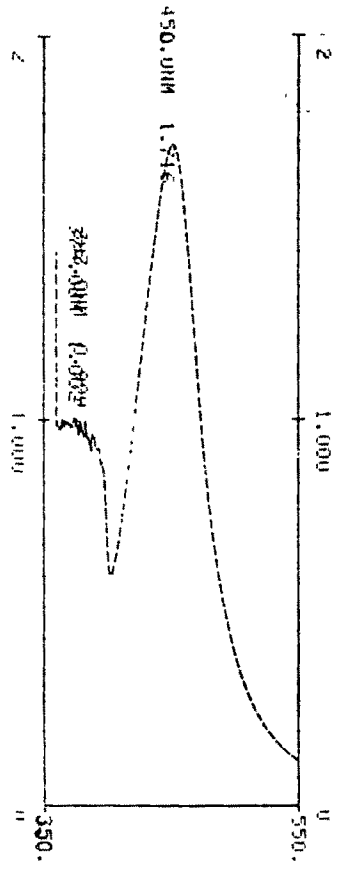


Fig. 2.4a : Absorption spectra of reaction product of DFS with DAB

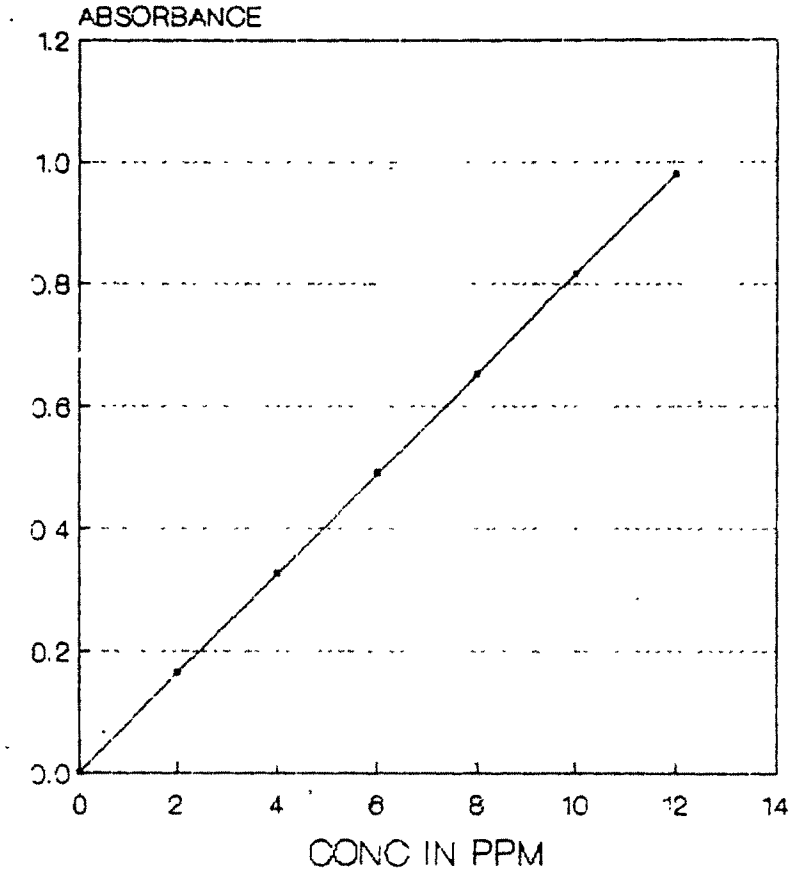


Fig. 2.4b : Linearity plot of DFS-DAB method

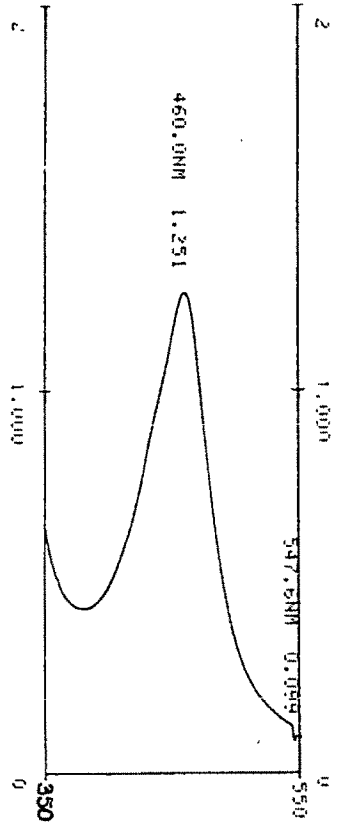


Fig. 2.4c : Absorption spectra of reaction product of DFS with SHB

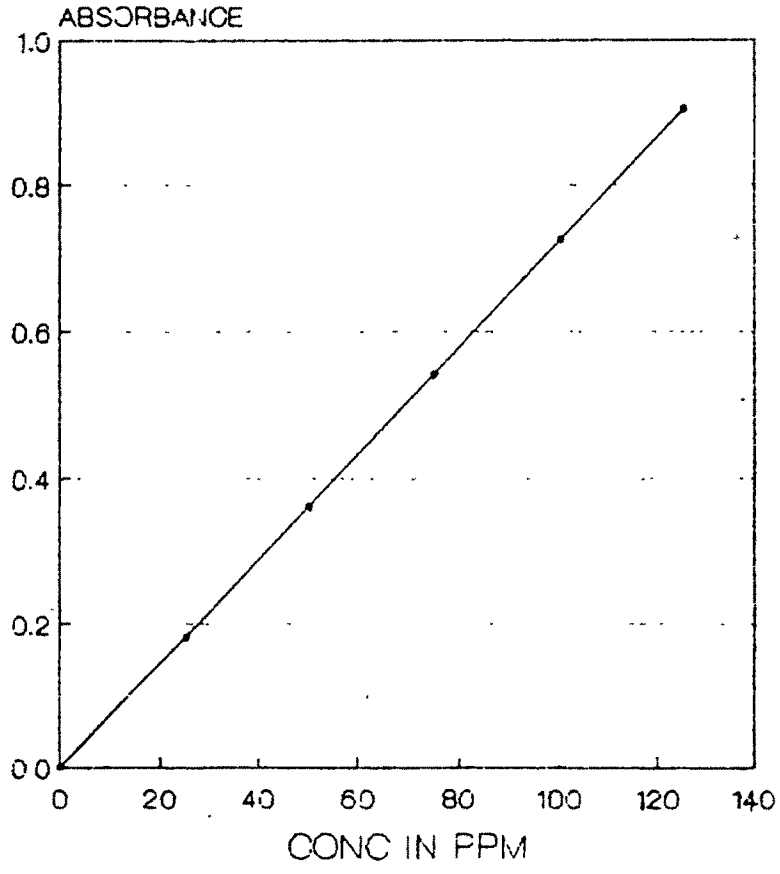


Fig. 2.4d : Linearity plot of DFS-SHB method

Table 2.8 : Results of diclofenac sodium tablet and injectable analysis using DAB and SHB.

Formulations	Labelled amount mg	% Found (± SD) DAB	% Found (± SD) SHB
Tablet A	50 per tab	98.36(± 0.26)	98.42(± 0.44)
Tablet B	50 per tab	99.21(± 0.32)	99.18(± 0.48)
Injectable	25 per ml	97.84(± 0.35)	97.89(± 0.38)

Several experiments were conducted to fix the optimum parameters like concentration of the reagent, order of addition, time for maximum colour development and reaction medium etc. The colour developed by both the reagents were stable for 40 minutes. Antioxidants included in the formulation were found to interfere in the determination. Hence extraction procedure was adapted in tablet analysis. Method using DAB is more sensitive as compared to SHB method. Intereference was observed in the presence of paracetamol in combination with DFS.

2.4 METHODS BASED ON REDOX REACTIONS

DFS reduces iron (III) when heated in aqueous solution. The ferrous ion produced reacts with 2,2'-bipyridine to form a complex having maximum absorbance at 520 nm.

Sodium nitroprusside reacts with FMD in alkaline media to form a nitroso derivative of FMD and in addition iron (III) in sodium nitroprusside is reduced to

iron (II). On acidification a red coloured complex formed with λmax of 498 nm.

2.4.1 PROCEDURES

Method A-7 for DFS: Aliqouts of aqueous DFS solution (1-5 ml, 200 $\mu g.ml^{-1}$) were placed into a series of 10 ml volumetric flasks. To each flask one ml of ferric chloride solution and 1 ml of 2,2'-bipyridine solutions were added. Each solution was diluted to 10 ml with distilled water. The flasks were stoppered and kept in a boiling water bath for 25 min. The solutions were cooled to room temperature and the absorbance was measured at 520 nm against a reagent blank.

For tablet and injectable analysis, a quantity of sample equivalent to 50 mg of the drug was placed in a 50 ml flask, 30 ml of distilled water was added and the solution was shaken for 5 min to dissolve the drug. The volume was made upto 50 ml and the solution was filtered. Five ml of filtrate was further diluted to 250 ml and 5 ml of solution was used for the colour development procedure.

Method C-4 for FMD: A standard solution containing 1 $mg.ml^{-1}$ of FMD was prepared in 0.1 M HCl. Into a series of 10 ml volumetric flasks aliquots of the drug solution in the range 1-5 ml were pippetted out. Volume was adjusted to 5 ml in all the flasks with 0.1 M HCl. To all the flasks 1 ml of 1N sodium hydroxide and 1 ml of sodium nitroprusside were added. After 2 min, 2 ml of 1 M hydrochloric acid was added and the volume was made upto 10 ml with distilled water. The absorbance values were measured at 498 nm against a reagent blank.

Tablet powder equivalent to 40 mg of the drug was transferred into a 100 ml beaker and 40 ml of 0.1 M HCl was added. The mixture was shaken for 5 min and filtered, the beaker was washed with 5x2 ml of 0.1 M HCl. The filtrate and washings were diluted to 100 ml with 0.1 M HCl. An aliquot of solution was

analysed for famotidine by the procedure described above.

2.4.2 RESULTS AND DISCUSSION

Method A-7 : Diclofenac sodium reduces iron(III) to iron(II) when heated in aqueous solution. The ferrous iron produced reacts with 2,2'-bipyridine to form a complex having maximum absorbance at 520 nm. The absorption spectrum of the coloured product is shown in Fig. 2.5. Spectral data for the reaction product and results of tablet analysis are given in Table 2.9.

Standardization of analytical parameters:

Volume of ferric chloride solution: To a fixed quantity of the drug solution (5 ml, 25 $\mu g.ml^{-1}$) different volumes (0.5 - 3.0 ml) of ferric chloride solution were added. The colour was developed as described under the procedure. It was found that an optimum volume of one ml of ferric chloride solution was required for maximum colour development.

Table 2.9 : Spectral characteristics and results of tablet analysis by bipyridine method.

Linearity range $\mu g.ml^{-1}$	Intercept	Correl-ation coeffi-cient	Molar absorp-tivity $L.mol^{-1}.cm^{-1}$	Amount found mg/tab*	S.D. %	% claim
10-80	0.0844	0.9992	3.965×10^3	49.73	0.47	99.46
				50.32	0.52	100.64
				48.77	0.55	97.54

* Labelled amount: 50mg/tablet.

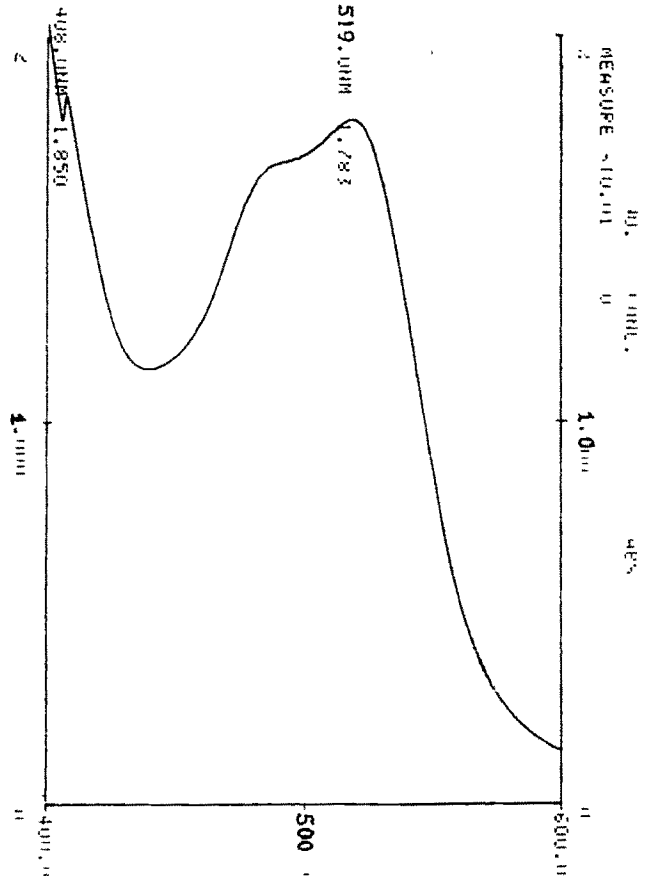


Fig. 2.5a : Absorption spectra of reaction product of DFS with iron(III) chloride and 2,2'-dipyridine

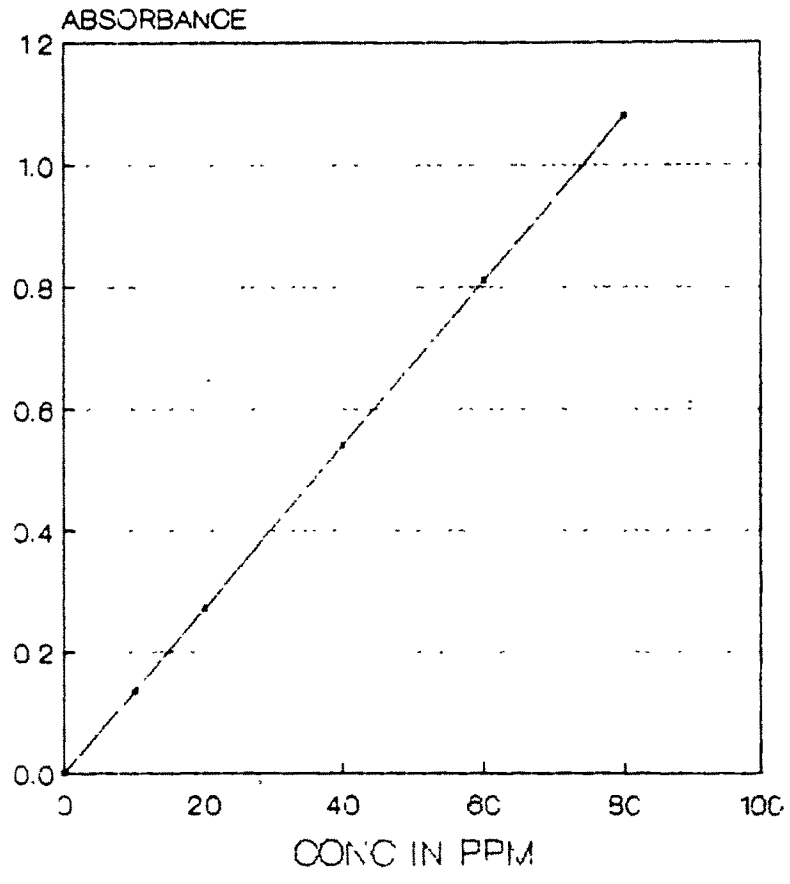


Fig. 2.5b : Linearity plot of DFS-BPN method

Volume of 2,2'-bipyridine solution: To a fixed quantity of the drug solution (5 ml, 25 $\mu g.ml^{-1}$) different volumes of (0.5-2.0 ml) of 2,2'-bipyridine solution were added and the colour was developed as described earlier. It was observed that an optimum volume of 1 ml of 2,2'-bipyridine solution was required for colour development.

Reproducibility and Recovery: Five ml of DFS solution (25 $\mu g.ml^{-1}$) was placed in each of six 10 ml flasks. The colour was develolped as described earlier. The absorbance values were found to be reproducible with RSD of 0.47%.

For recovery studies 3 ml of standard DFS solution was added to 3 ml of tablet solution which had been analysed earlier. The colour of this solution was developed together with that of 6 ml of standard DFS solution. The recovery of the drug was 98.92-101.03%.

The method described is simple and sensitive. Common excipients found in tablet preparation do not interfere. Interference was observed in the presence of oxidising agents but these can be removed by a suitable extraction procedure. The method is not suitable for the determination of DFS in presence of paracetamol since ferric chloride will react with paracetamol.

Method C-4: Sodium nitroprusside reacts with FMD in alkaline medium to form a nitroso derivative of famotidine and in addition iron(III) in sodium nitroprusside is reduced to iron(II). The red coloured complex formed on acidification of

solution had a λ max of 498 nm (Fig 2.6) a molar absorptivity of 5.9 x 10^2 $L.mol^{-1}.cm^{-1}$ and a Sandell's sensitivity of 0.572 $\mu g.cm^{-2}$. Beer's law was obeyed in the range 50-500 $\mu g.ml^{-1}$ of FMD. The regression equation was: conc = 563.67 x absorbance - 1.78, (correlation coefficient = 0.999).

The effect of changes in the concentration of hydrochloric acid was studied for a fixed concentration of 500 $\mu g.ml^{-1}$ of FMD. A 2 ml volume of 1 M hydrochloric acid was adequate for maximum colour development (Table 2.10).

Table 2.10 : Effect of HCl molarity on colour development of FMD with SNP.

HCl molarity	Volume added(ml)	Absorbance	Molar absorptivity $L.mol^{-1}.cm^{-1}$ ($x10^{-2}$)
0.25	2	0.280	1.0
0.50	2	0.695	4.6
0.75	2	0.735	4.6
1.00	1	0.700	4.8
1.00	2	0.888	5.9
1.00	3	0.887	5.9
1.00	4	0.888	5.9

* Famotidine: 500 µg, λ max 498 nm.

Higher HCl concentration did not adversely affect colour development. However, lower HCl concentration decreased the absorbance value. Similarly an optimum volume of 1 ml of 1% sodium nitroprusside solution was required for the working concentration range (50-500 µg) of famotidine (Table 2.11)

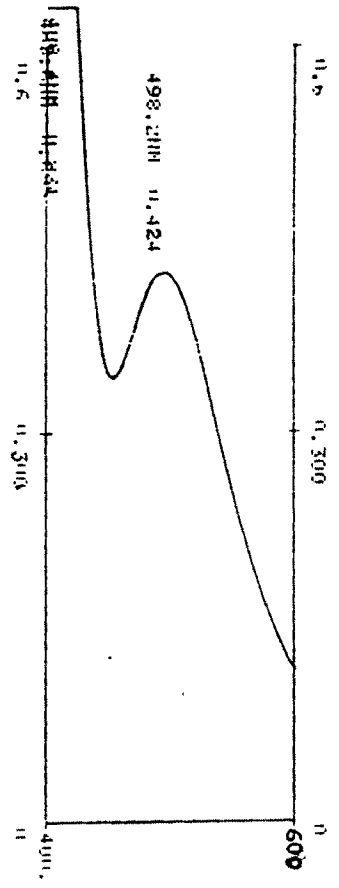


Fig. 2.6a : **Absorption spectra of reaction Product of FMD with SNP**

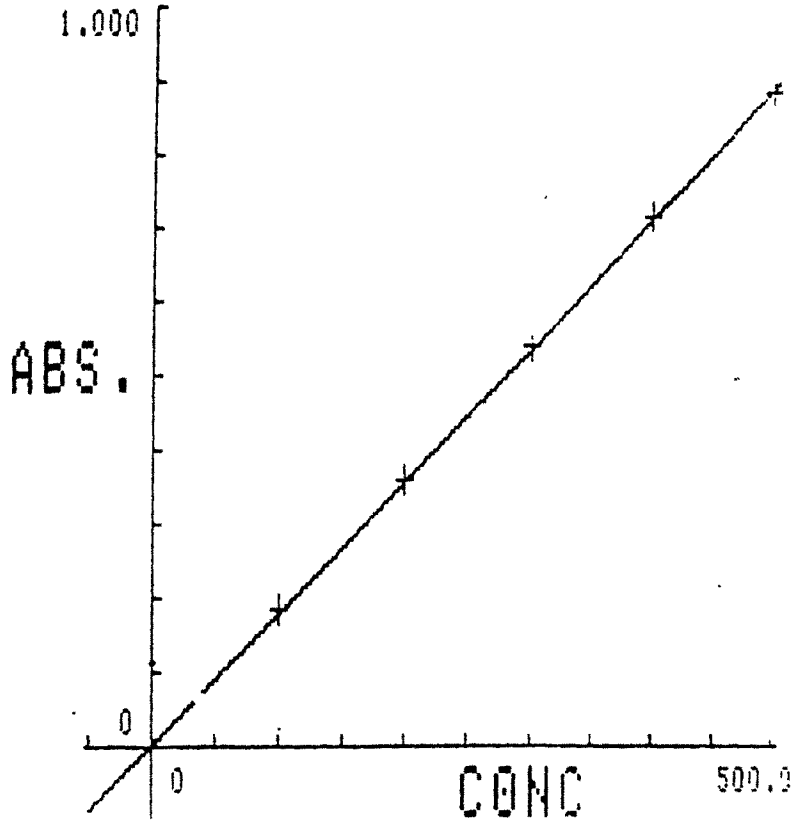


Fig. 2.6b : **Linearity plot of FMD-SNP method**

Table 2.11 : Effect of sodium nitroprusside concentration on colour development of FMD.

Sodium nitroprusside(1%) (ml)	Absorbance	Molar absroptivity $L.mol^{-1}.cm^{-1}(x10^{-2})$
0.2	0.050	0.3
0.4	0.100	0.7
0.6	0.500	3.3
0.8	0.780	5.2
1.0	0.888	5.9
1.5	0.888	5.9
2.0	0.888	5.9

* Famotidine: 500 µg,1 M HCl,2 ml, λ max 498 nm.

The order of addition of the reagent proposed is: first, sodium nitroprusside should be treated with famotidine in alkaline condition; 2 ml of 1 M HCl is then added to make the solution acidic. The colour remained stable for more than 1 hour.

Table 2.12: Analysis of famotidine tablets by SNP method.

Labelled amount ($mg.tablet^{-1}$)	Famotidine found (mg)		
	SNP method		USP method
	Mean	S.D.	
20	19.95	0.2	19.96
20	19.55	0.5	19.57
40	39.92	0.2	39.40
40	39.96	0.1	39.80
40	40.10	0.1	40.15

* SNP 1 ml 1%, 1 M HCl 2 ml, λmax 498nm..

The reproducibility of the method was found to be satisfactory with a relative standard deviation of 1.5% (n = 6). The mean recovery of added famotidine to pre-analysed formulation was found to be 100.26% (n = 6). The tablet analysis given in Table 2.12 are comparable with those obtained by the USP method. Common excipients did not interfere in this method (Table 2.13).

Table 2.13 : Analysis of famotidine in the presence of various excipients in synthetic mixtures.

Excipients	Recovery (%)
Talc	99.72
Propylene glycol	99.76
Magnesium stearate	99.54
Starch	100.35
Lactose	99.80
Acacia	100.50
Tragacanth	99.99
Glycerine	99.80

* sodium nitroprusside 1 ml 1 %, 1 M HCl 2ml.

2.5 METHODS BASED ON ION-PAIR COMPLEXATION REACTIONS

Extractive spectrophotometric methods were developed for the determination of DFS in pharmaceutical formulations. The methods are based on the formation of ion-pair complexes of the drug with reagents like acridine orange, basic fuchsin, methylene blue, safranine and toluidine blue in presence of phosphate buffer. The complexes formed were extracted into chloroform and the absorbance was measured at wavelength of maximum absorption. DLZ forms coloured ion-pair complexes with acidic dyes like bromothymol blue, bromocresol green, bromocresol

purple, bromophenol blue, eriochrome black T, methyl orange, picric acid, solochrome dark blue and tropaeolin 00 in acidic medium. The complexes formed were quantitatively extracted in chloroform and absorbance was measured at appropriate λmax. KTR was analysed in ion-pair complexation with methylene blue and safranine.

2.5A.1 PROCEDURES:

Methods A-8 to A-12 for DFS: Into a series of 125 ml separating funnels different volumes of drug solutions in the range (60 $\mu g.ml^{-1}$, 1-10ml) were pipetted. Total volume was adjusted to 10 ml with appropriate buffer shown in Table 2.14. To each funnel 5 ml of the dye solution and 20 ml of chloroform were added. The solutions were shaken for 2 min and allowed to stand for clear separation of two layers. The chloroform layer was passed through anhydrous sodium sulphate and the absorbance was measured at wavelength of maximum absorption against a reagent blank.

Tablet powder equivalent to 50 mg of DFS was shaken with 100 ml of water and filtered. Five ml of filtrate was further diluted to 50 ml with appropriate buffer. Five ml of solution was used for the colour development according to the method described above. The amount of the drug corresponding to the absorbance value was found out from the calibration graph.

2.5A.2 RESULTS AND DISCUSSION

Diclofenac sodium was found to form ion-pair complexes with basic dyes like acridine orange (AO), basic fuchsin (BF), methylene blue (MB), safranine (SF) and toluidine blue (TB) in presence of phosphate buffer. The complexes formed are extracted in chloroform and the absorbance was measured at wavelength of

maximum absorption. Optical characteristics and results of tablet analysis are given in Table 2.14. Absorption spectra of ion-pair complexes are shown in Fig. 2.7.

Table 2.14 : Results of determination of DFS.

Reagent	pH	λ max nm	Linearity range $\mu g.ml^{-1}$	Molar absorptivity $L.mol^{-1}.cm^{-1}$	Amount found mg/tab*	SD	Recovery %
AO	6.8	470	2-15	$2.04x10^4$	49.75	0.86	99.42
BF	7.0	556	2-18	$1.80x10^4$	49.62	0.40	100.16
MB	6.8	640	3-20	$1.49x10^4$	49.65	0.28	98.96
SF	7.4	515	3-17	$1.84x10^4$	49.68	0.21	99.15
TB	6.8	630	3-30	$1.11x10^4$	49.60	0.36	99.20

Labelled amount: 50 mg/tablet

Standardization of analytical parameters:

Dye concentration: To a fixed quantity of the drug solution (5 ml, 60 $\mu g.ml^{-1}$) different volumes (1-7 ml) of the dye solution were added in separating funnels. The volume of the total aqueous layer was adjusted to 15 ml with buffer solution. The complex was extracted with 20 ml of chloroform and the absorbance was measured. An optimum volume of 5 ml of dye solution was required for maximum colour development.

pH of buffer: Five ml of standard drug solution in water (30 $\mu g.ml^{-1}$) were taken in a series of separating funnels. Ten ml of 0.1 M phosphate buffer of different pH (5.5, 6.0, 6.5, 6.8, 7.0, 7.4, 8.0) were added and the colour of the chloroform extract was measured against reagent blank. Maximum colour was observed at the pH specified in the Table 2.14. The concentration of the buffer was fixed at 0.1 M after several experiments.

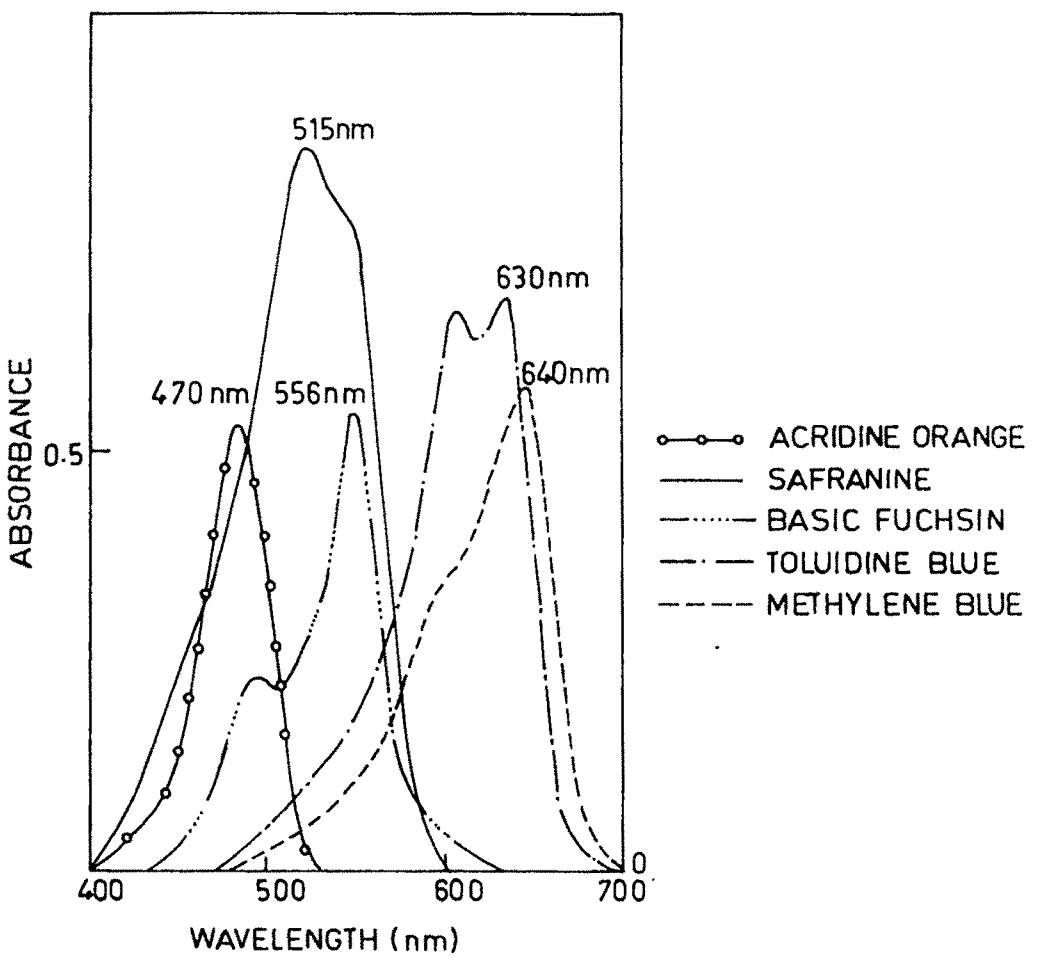


Fig. 2.7a : Absorption spectra of ion pair complexes of DFS with basic dyes

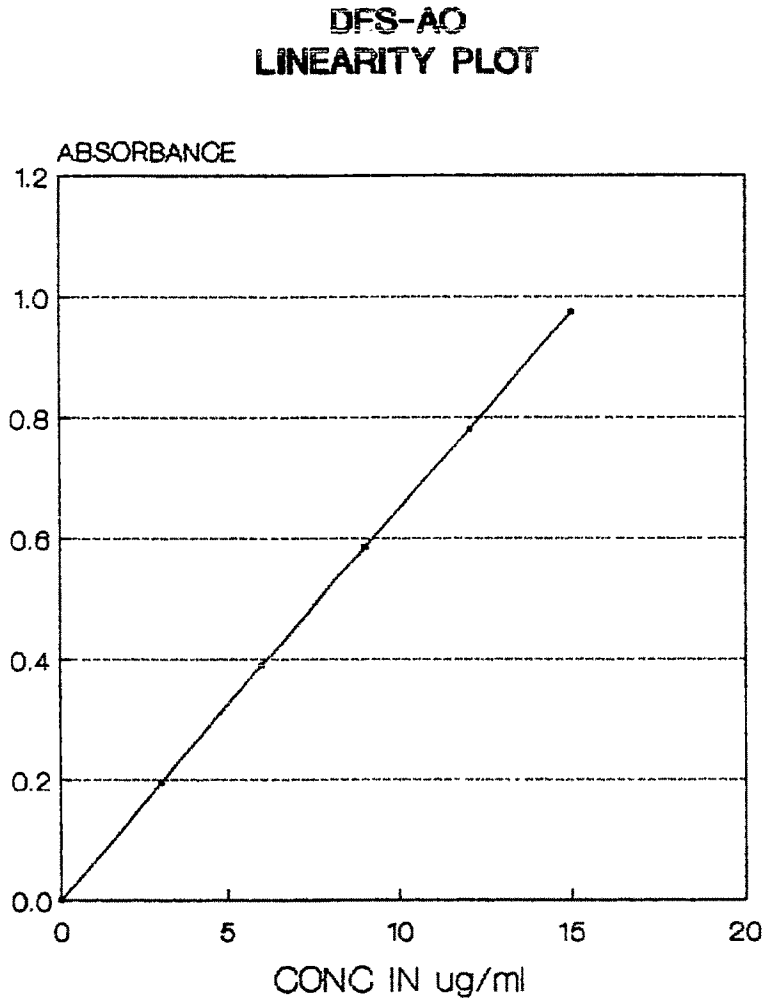


Fig. 2.7b : Linearity plot of DFS-AO method

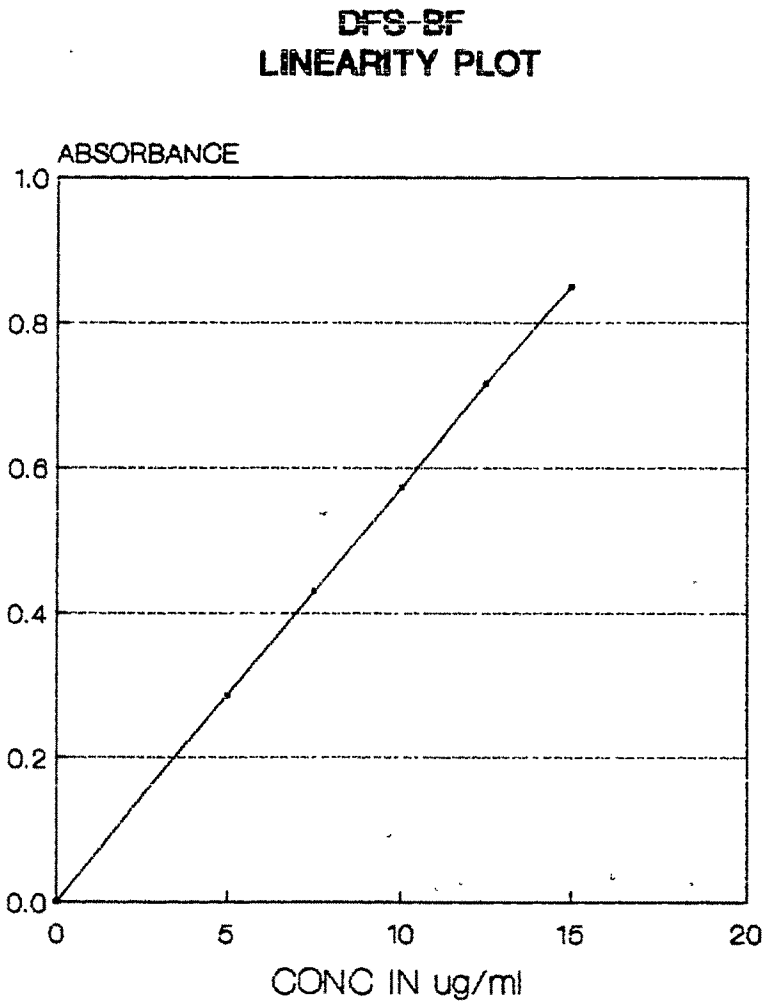


Fig. 2.7c : Linearity plot of DFS-BF method

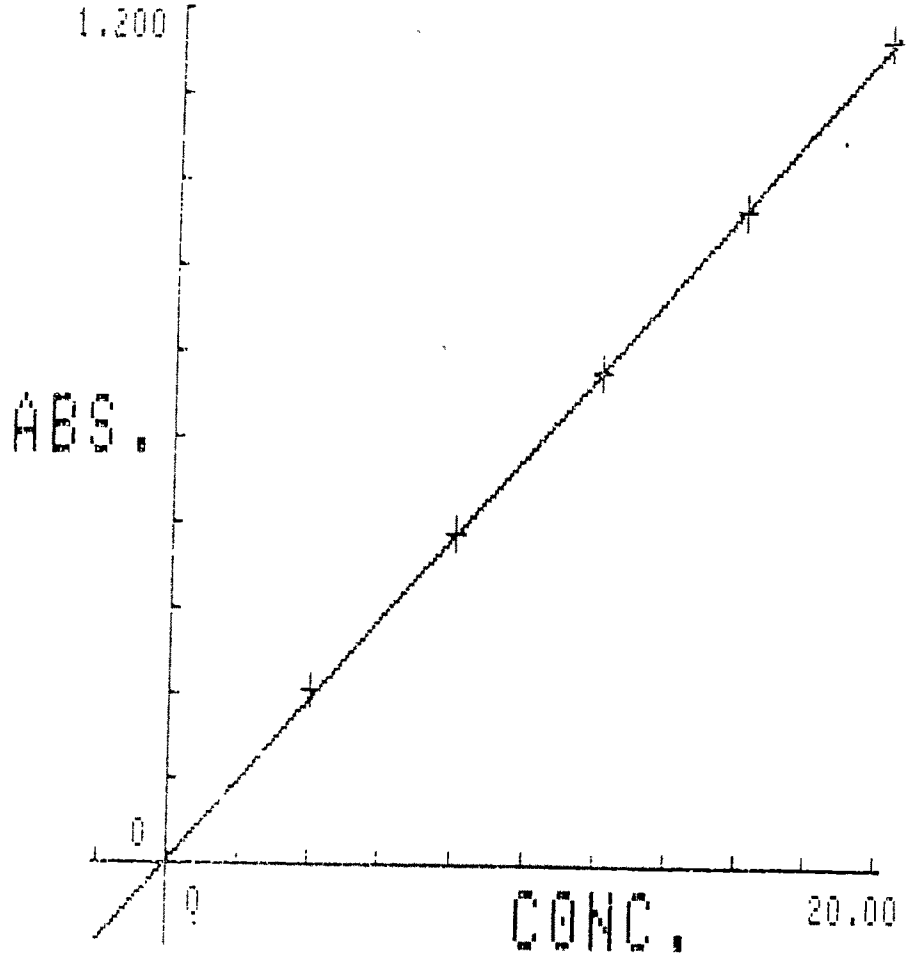


Fig. 2.7d : Linearity plot of DFS-SF method

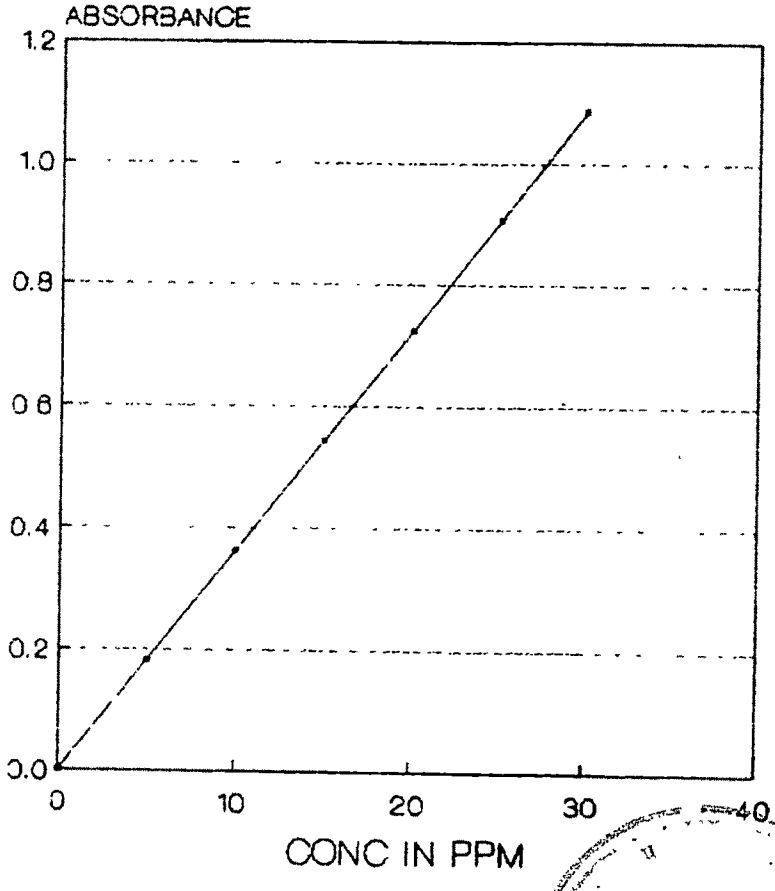


Fig. 2.7e : Linearity plot of DFS-TB method

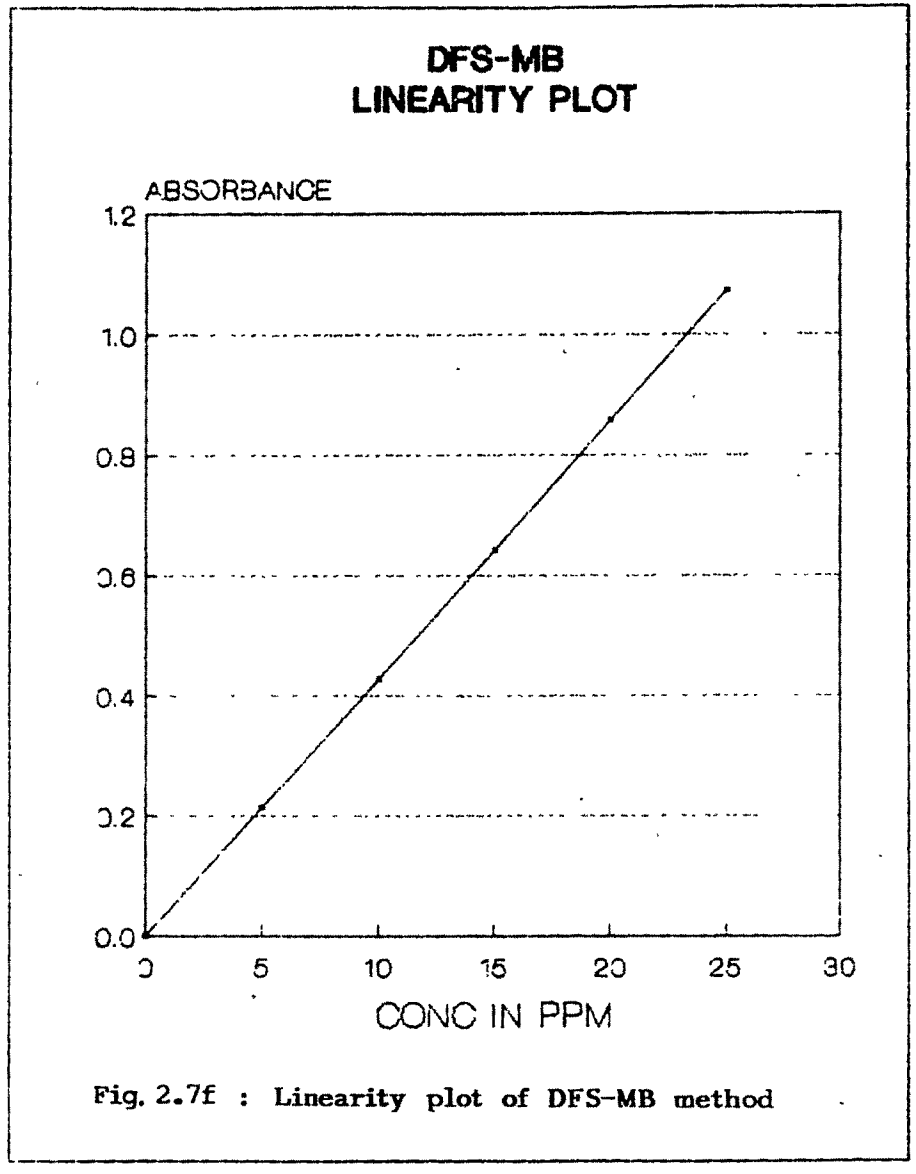


Fig. 2.7f : Linearity plot of DFS-MB method

Volume ratio: After several sets of extraction experiments it was found that the extraction was quantitative when the ratio (v/v) of aqueous solution to chloroform was 15 : 20.

Mole ratio: The molar ratio of the drug to reagent in the complex as determined by continuous variation method[82] was 1 : 1 in all the cases.

2.5B.1 PROCEDURES

Methods B-3 to B-11 for DLZ: Into a series of 125 ml separating funnels different volumes of aqueous solution of DLZ (250 $\mu g.ml^{-1}$, 1-5 ml) were pipetted out. The total volume was adjusted to 5 ml in all the funnels with distilled water. To each funnel 5 ml of buffer (Table 2.15) and 5 ml of dye were added. Twenty five ml of chloroform was added to all the funnels to extract the ion-association complex. The solutions were shaken for 2 min and allowed to stand for clear separation of the two layers. The lower chloroform layer was passed through anhydrous sodium sulphate and the absorbance was measured at respective wavelength of maximum absorption (Table 2.15) against reagent blank.

For tablet analysis, powdered tablet equivalent to 30 mg of the drug was shaken with 250 ml of water and filtered. Five ml of the filtrate was used for the colour development by the method described above.

2.5B.2. RESULTS AND DISCUSSION

Diltiazem hydrochloride being a basic compound reacts with acid dyes forming neutral complexes which are extractable in chloroform. At specified pH of the buffer, neither the drug nor the dye are extracted into chloroform to give coloured products. However, upon mixing both the reactants they produce a highly coloured product which can be extracted into chloroform. The absorbance was

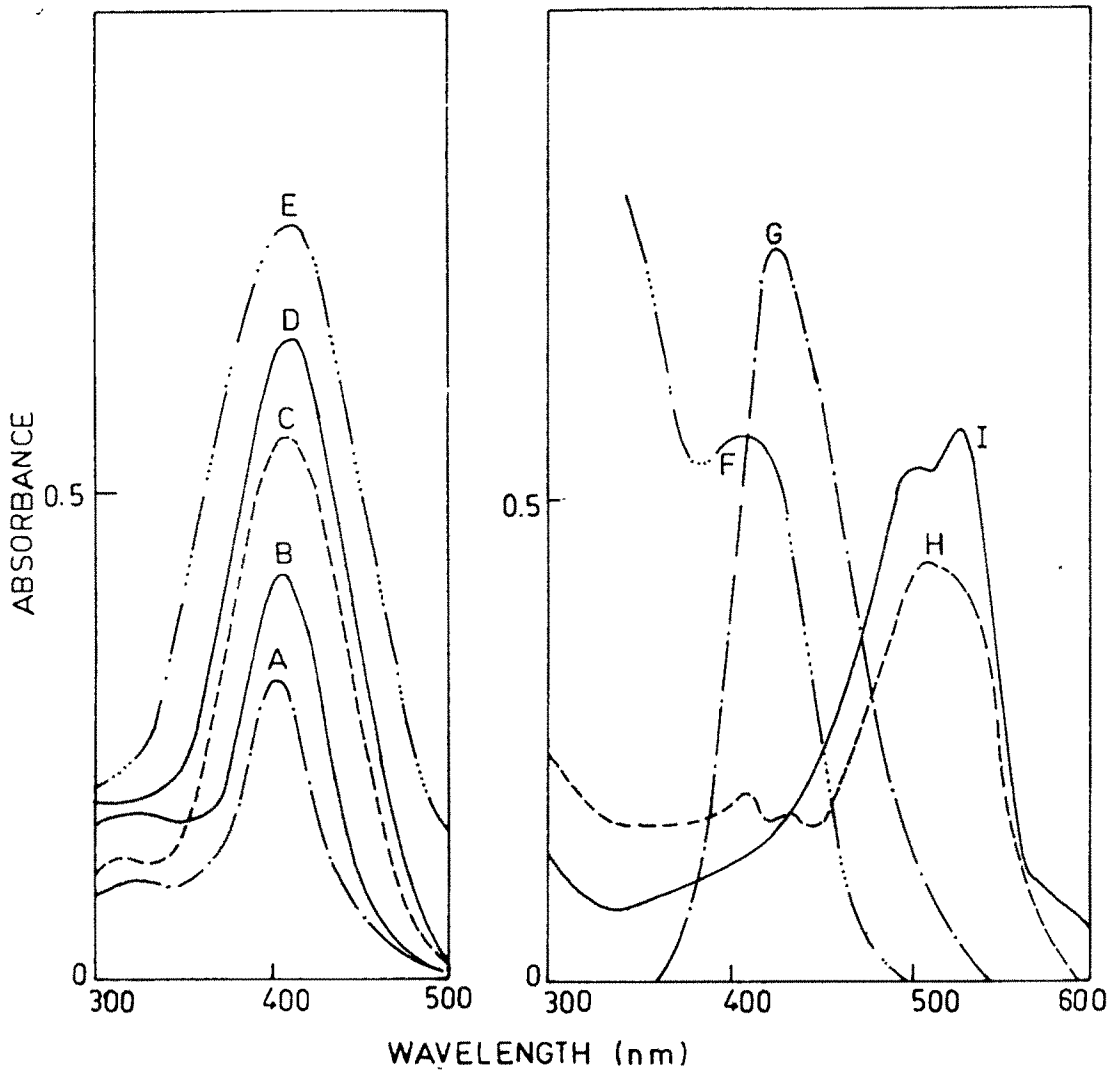
measured at wavelength of maximum absorption. Absorption spectra of ion-pair complexes of DLZ with acid dyes are shown in Fig. 2.8. Spectral characteristics and the results of tablet analysis are summarized in Table 2.15.

Several experiments were conducted to fix the optimum parameters like concentration of the dye, pH of the medium, ratio of aqueous to nonaqueous layer. It was found that 5 ml of 0.1 % dye solution is optimum for maximum colour development in the drug concentration range studied. Among the various organic solvents viz, chloroform, dichloromethane, benzene, toluene and hexane used for extraction of the complex, chloroform gave satisfactory result. The colour developed is stable for more than 4 hours. The molar ratio of drug/dye in the complex was found to be 1 : 1 by continuous variation method[82]. Twenty five ml of chloroform is sufficient to extract the complex from 15 ml of aqueous solution.

The methods described are simple and rapid. The percent recovery values are in the range 98 to 100% which indicates non-interference by excipients present in the formulations. However, interference was observed from desacetyl diltiazem if present in dosage form. Recovery values and standard deviation are satisfactory. The proposed methods can be used for the routine determination of DLZ from tablets.

Table 2.15 : Results of determination of Diltiazem hydrochloride.

Reagent	pH	λmax (nm)	Linearity range $\mu g.ml^{-1}$	Molar absorptivity $L.mol^{-1}.cm^{-1}$	%Recovery	SD	Amount	
							Labelled $mg.tab^{-1}$	Found $mg.tab^{-1}$
MTO	4.5	420	5-50	$0.84x10^4$	98.26	0.67	30	29.34
					98.65	0.52	60	59.34
EBT	5.0	510	4-40	$1.01x10^4$	98.72	0.56	30	29.62
					98.75	0.68	60	60.01
TPO	2.5	410	5-40	$0.95x10^4$	98.56	0.86	30	29.58
					98.88	0.70	60	59.82
PCA	2.5	404	5-50	$0.82x10^4$	98.50	0.42	30	29.68
					99.12	0.36	60	59.86
BCG	2.5	410	3-25	$1.77x10^4$	98.74	0.64	30	29.32
					99.45	1.82	60	59.65
BCP	4.7	405	2-20	$2.15x10^4$	98.62	0.52	30	29.87
					99.94	1.24	60	60.13
BPB	5.0	410	3-25	$1.98x10^4$	98.86	0.46	30	29.65
					99.42	1.78	60	59.78
BTB	2.5	410	3-25	$1.82x10^4$	99.95	0.76	30	30.14
					99.73	0.98	60	59.96
SDB	1.5	530	5-30	$1.45x10^4$	99.86	1.12	30	30.06
					99.90	1.68	60	60.28



A : Bromo cresol purple (405 nm)
B : Bromo cresol green (410 nm)
C : Bromo phenol blue (410 nm)
D : Bromo thymol blue (410 nm)
E : Tropaeolin 00 (410 nm)
F : Picric acid (404 nm)
G : Methyl orange (420 nm)
H : Eriochrome balck T. (510 nm)
I : Solochrome dark blue (530 nm)

Fig. 2.8 : Absorption spectra of ion-pair Complexes of DLZ with acid dyes

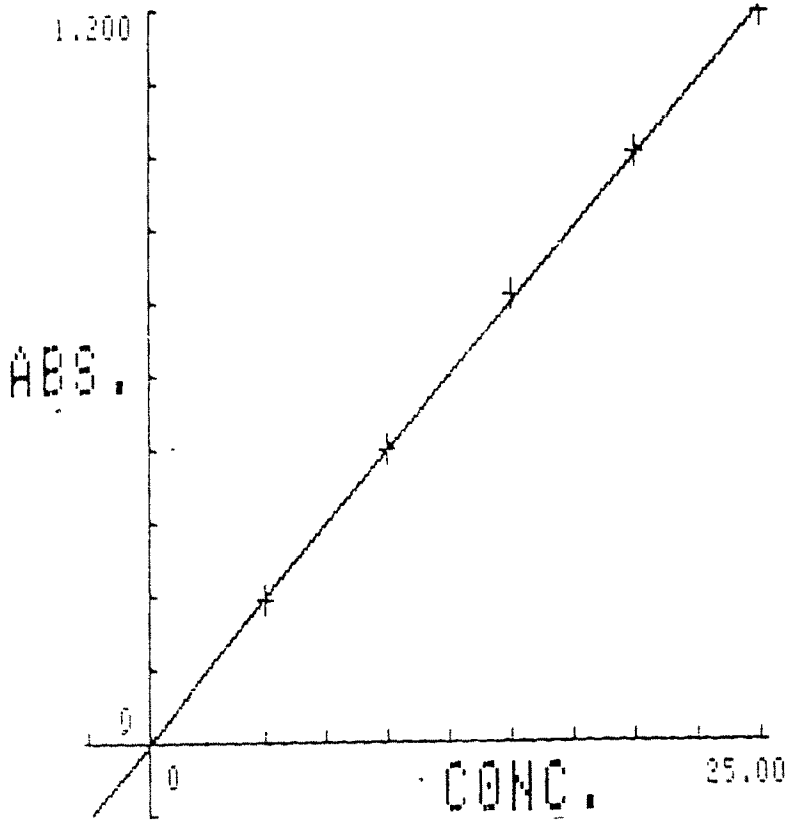


Fig. 2.8a : Linearity plot of DLZ-BCP method

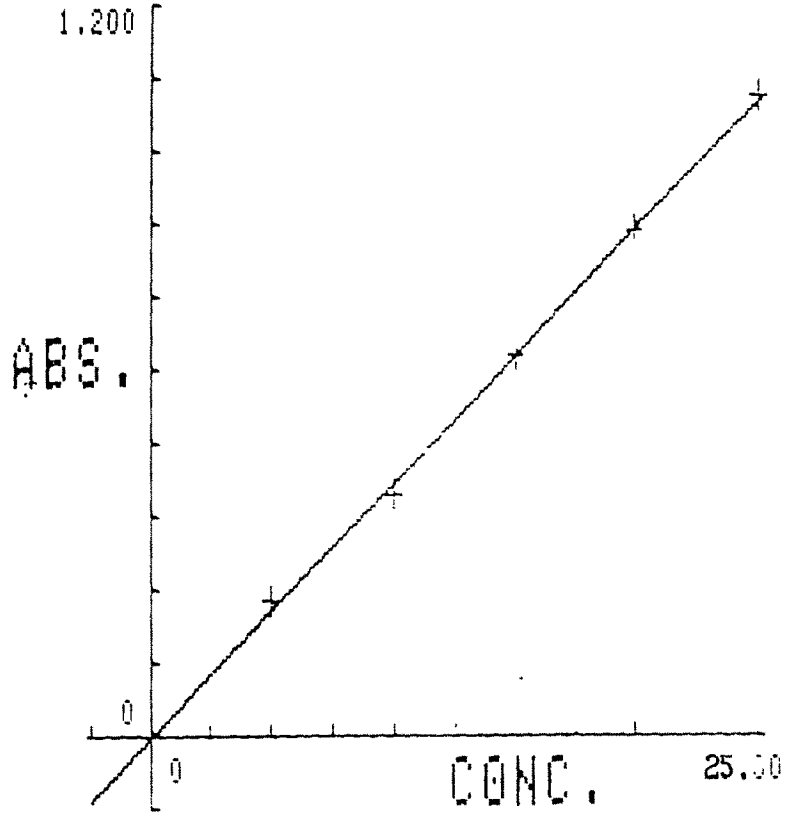


Fig. 2.8b : Linearity plot of DLZ-BCG method

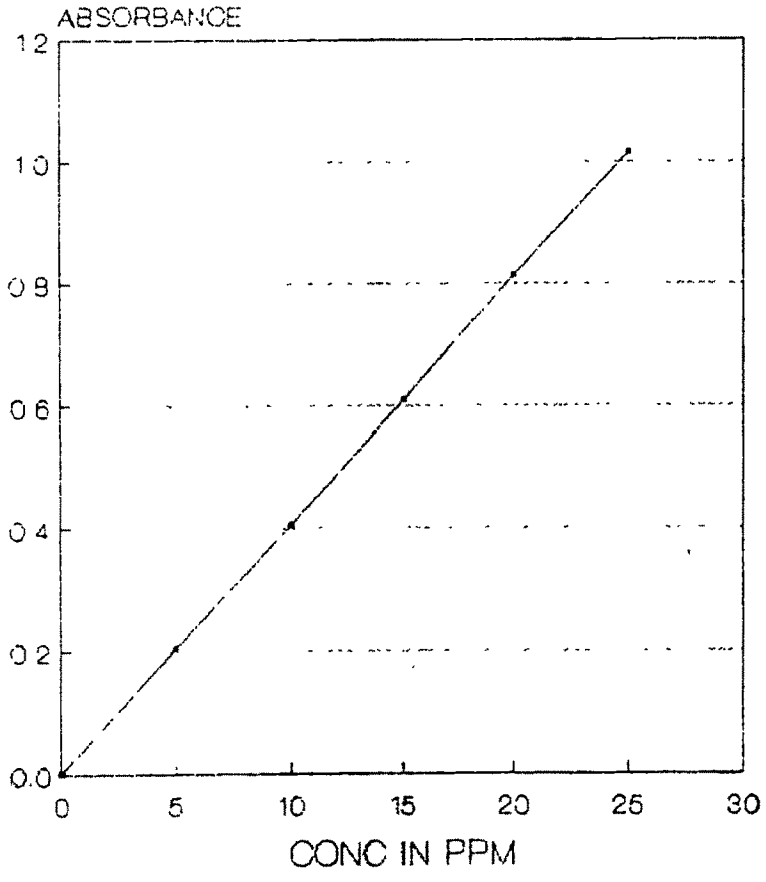


Fig. 2.8c : Linearity plot of DLZ-BPB method

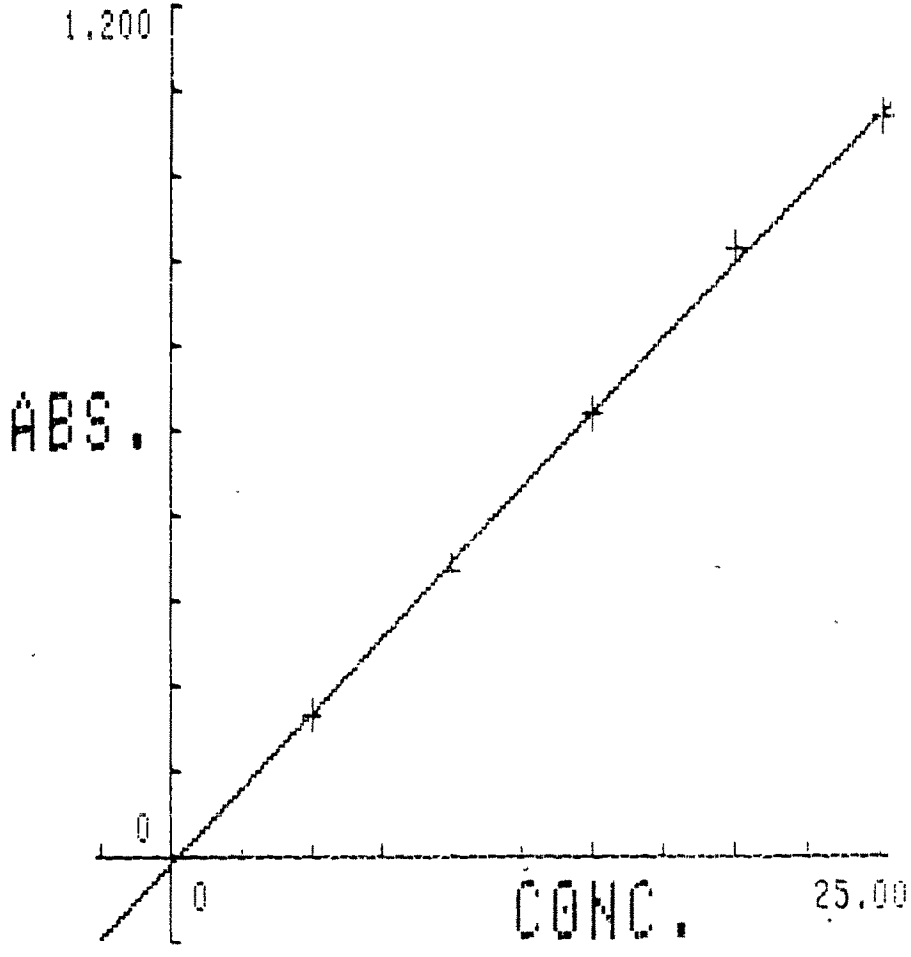


Fig. 2.8d : Linearity plot of DLZ-BTB method

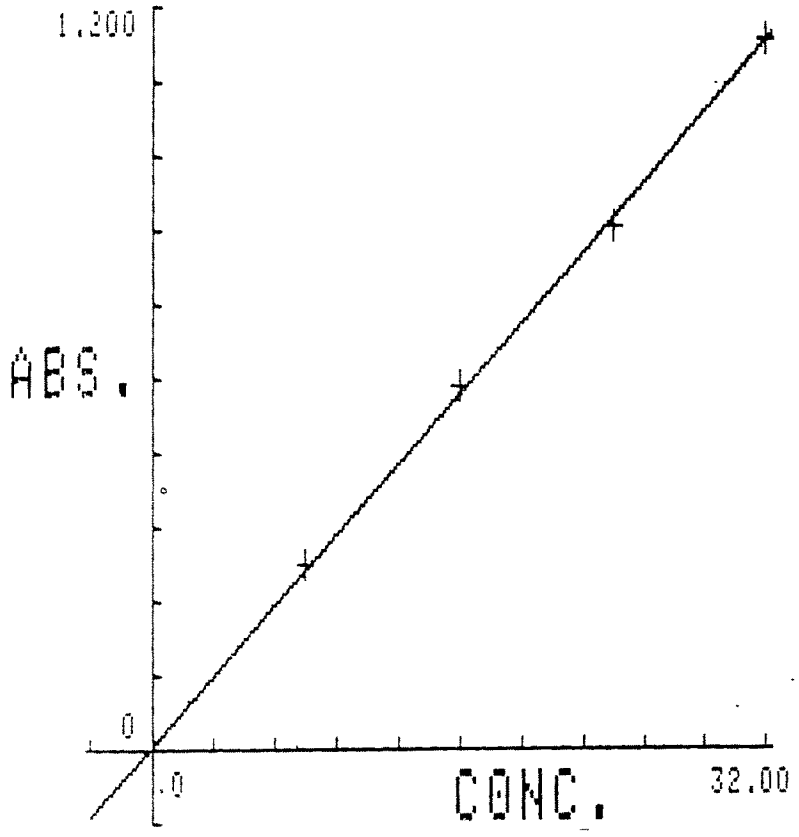


Fig. 2.8e : Linearity plot of DLZ-TPO method

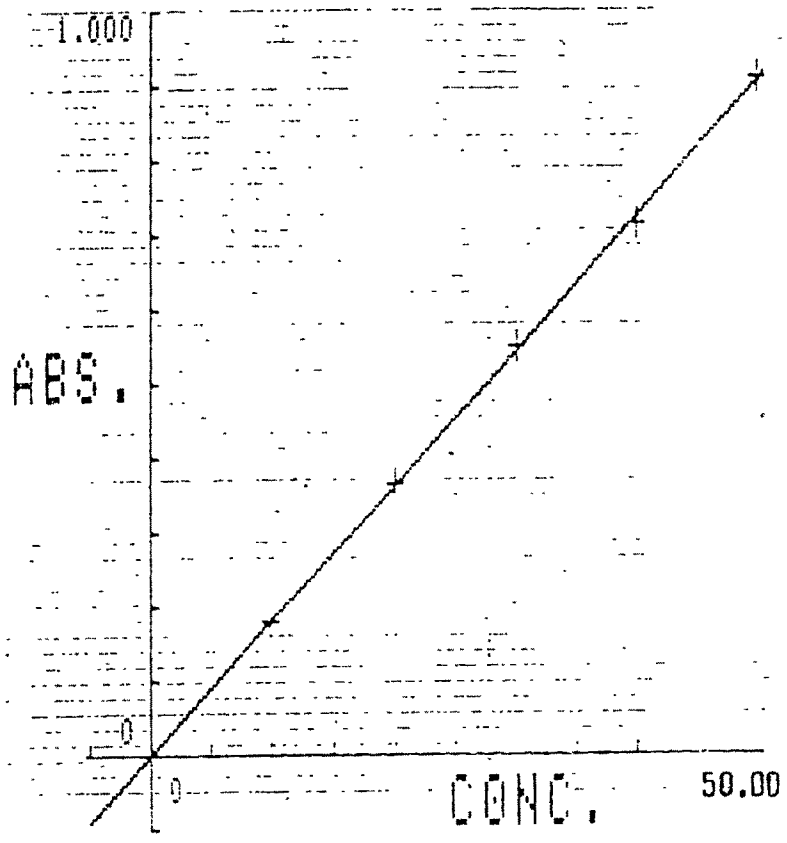


Fig. 2.8f : Linearity plot of DLZ-PCA method

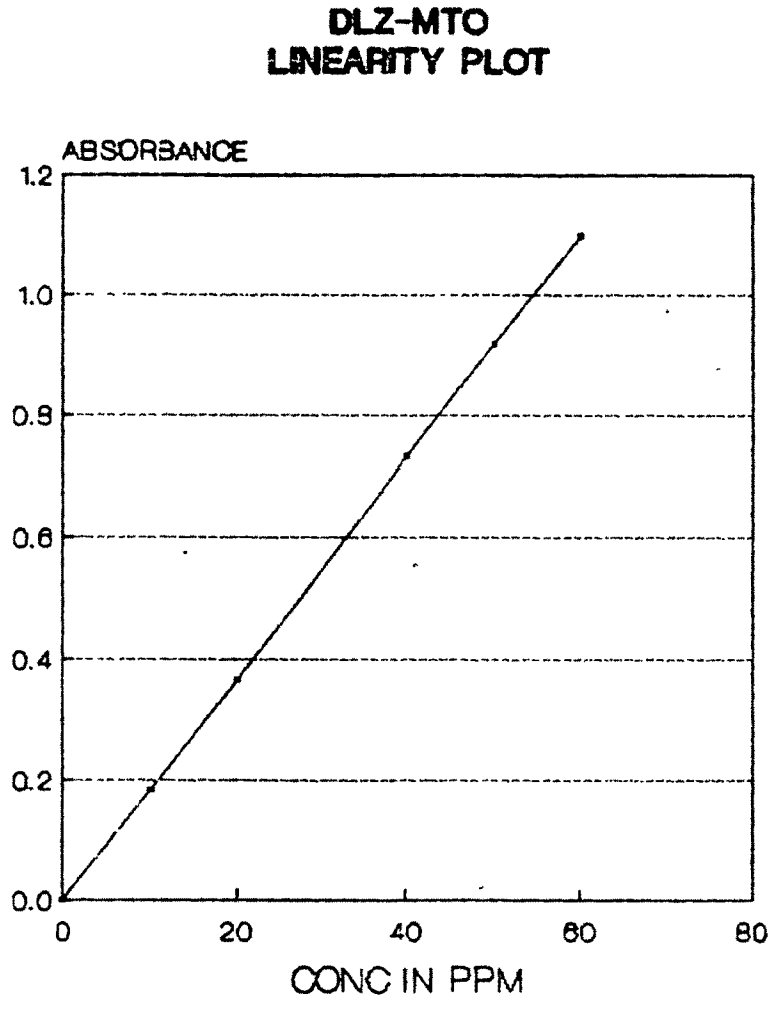


Fig. 2.8g : Linearity plot of DLZ-MTO method

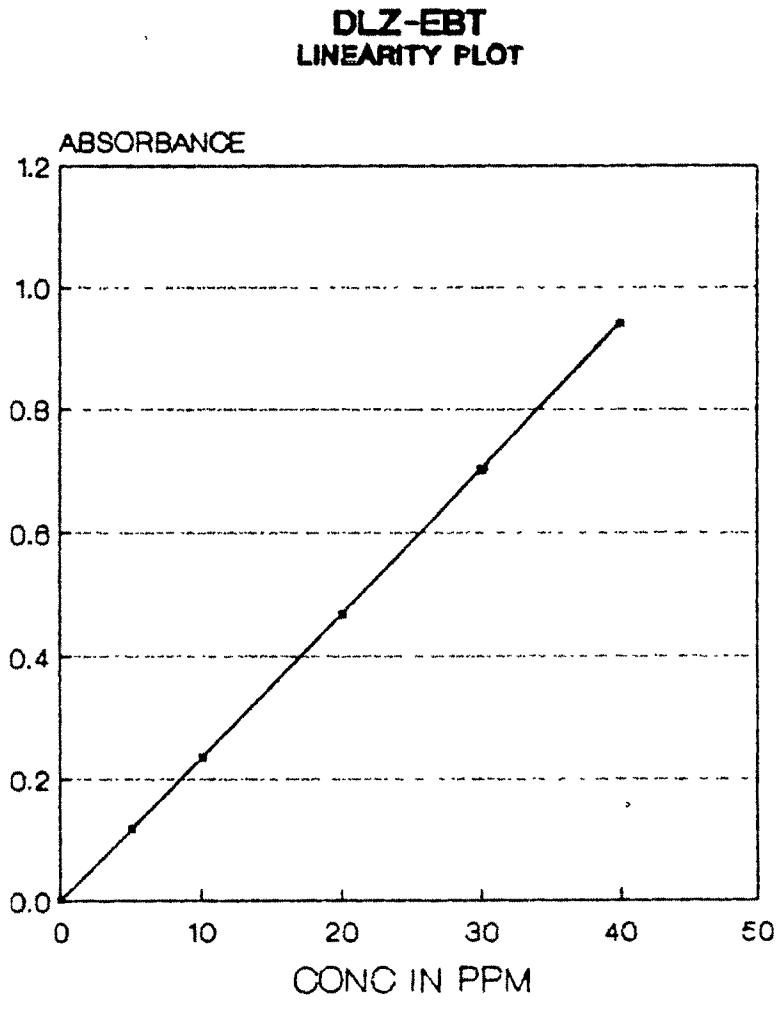


Fig. 2.8h : Linearity plot of DLZ-EBT method

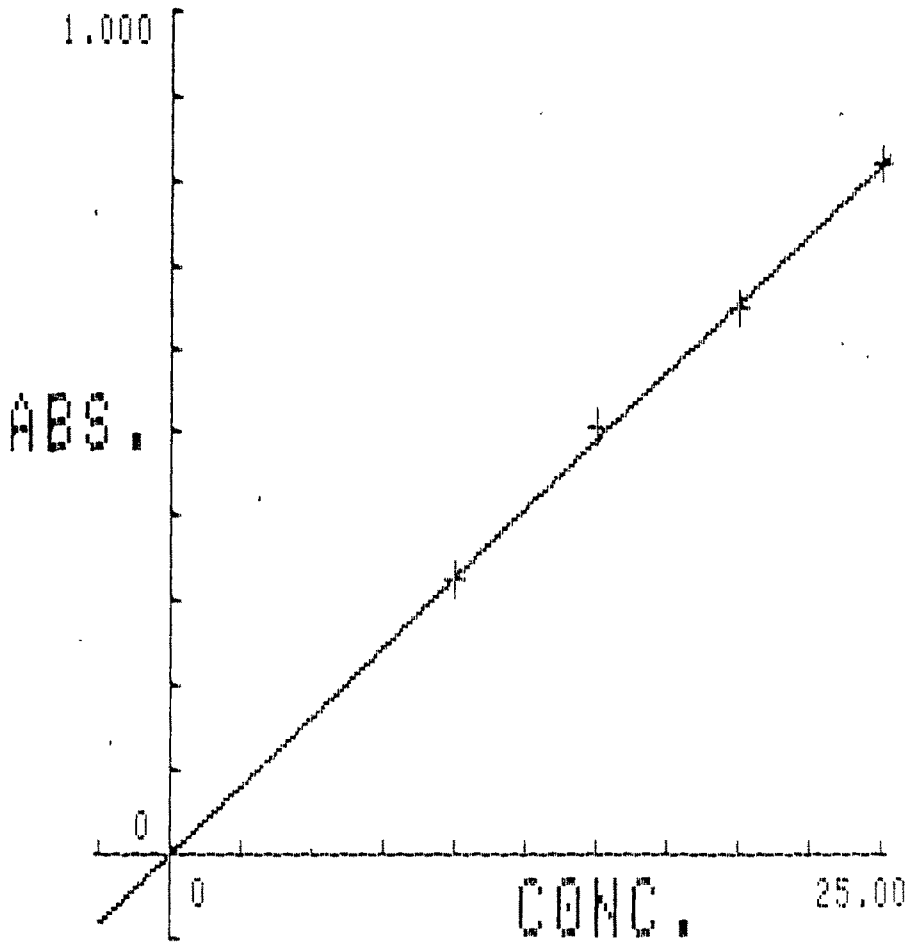


Fig.2.8i : Linearity plot of DLZ-SDB method

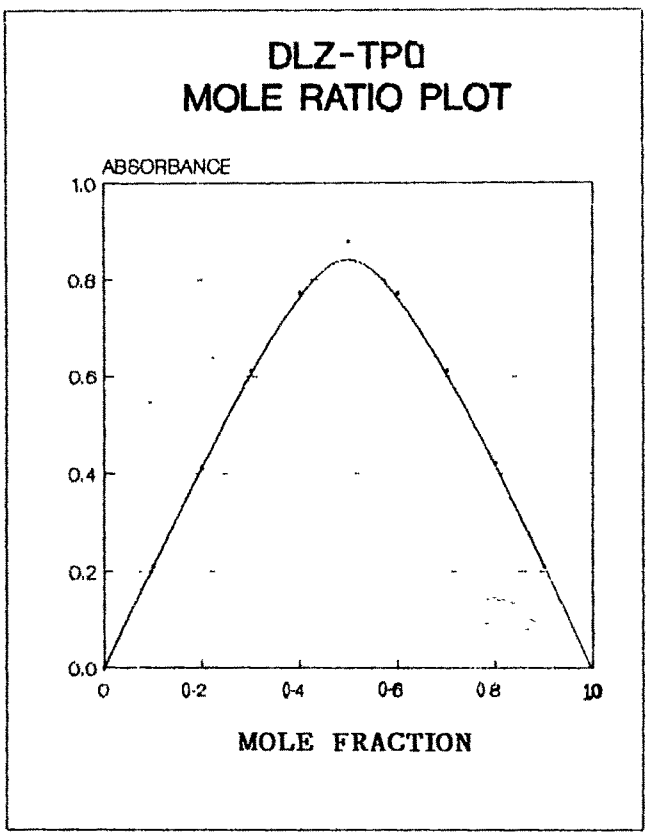


Fig. 2.8j : Continuous variation plot of DLZ-TPO complex

2.5C.1 PROCEDURES

Methods D-1 and D-2 for KTR: Aliquots of standard stock solution in water (250 $\mu g.ml^{-1}$, 0.5-5.0 ml) were placed in a series of 125 ml separating funnels. The total volume was adjusted to 5 ml in all the funnels using distilled water. To each funnel 5 ml of phosphate buffer (Table 2.16) and 5 ml of methylene blue or safranine solutions were added and the complex was extracted with 10, 10 and 5 ml portions of chloroform. The chloroform layers were mixed and passed through anhydrous sodium sulphate (0.5 g) and collected in 25 ml volumetric flask. The absorbance was measured at λmax against reagent blank within 20 min.

For formulation analysis, weight of powdered tablet equivalent to 25 mg KTR or volume of injectable equivalent to 30 mg KTR was diluted to 200 ml with water in a volumetric flask. The contents were stirred for 10 min and filtered. Five ml of the filtrate was used for the method described above. The amount of the drug corresponding to the absorbance value was found out from the calibration graph. The content of the drug per dosage form was calculated using the dilution factor.

2.5C.2 RESULTS AND DISCUSSION

KTR reacts with basic dyes like methylene blue and safranine in presence of phosphate buffer (pH = 6.8, 7.4) forming neutral complexes which are extractable in chloroform. Fig. 2.9 shows the absorption spectra of coloured products with λmax at 640 nm for methylene blue and 515 nm for safranine. Spectral data for these ion-pair complexes are given in Table 2.16.

Table 2.16: Spectral data for the reaction products of KTR with MB and SF.

Reagent	pH	λmax nm	Linearity range $\mu g.ml^{-1}$	Molar absorptivity $L.mol^{-1}.cm^{-1}$	Intercept	Correlation coefficient
MB	6.8	640	5-50	0.762×10^4	0.0069	0.9995
SF	7.4	515	5-50	0.771×10^4	0.0087	0.9996

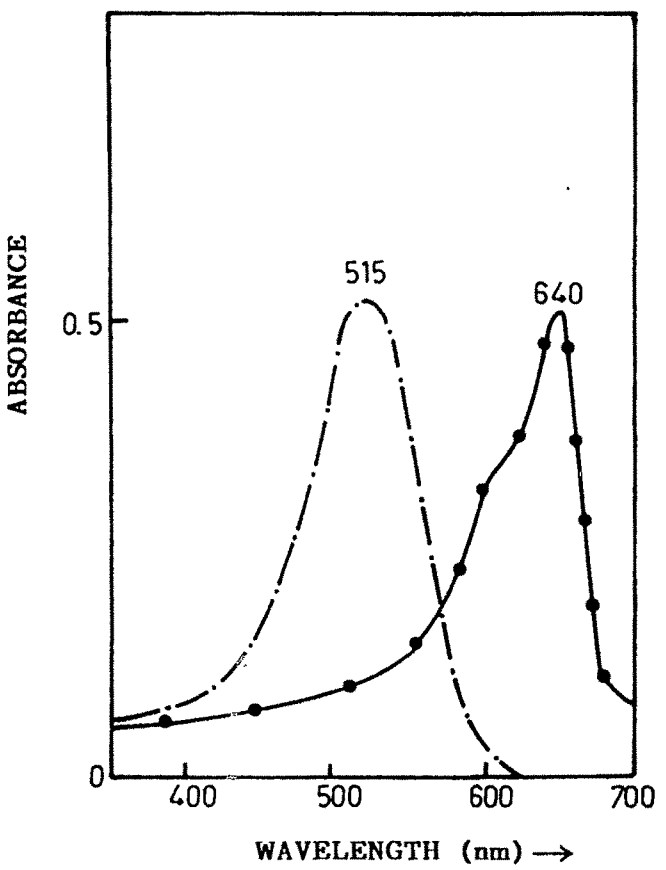


Fig. 2.9a : Absorption spectra of ion pair complexes of KTR with MB and SF

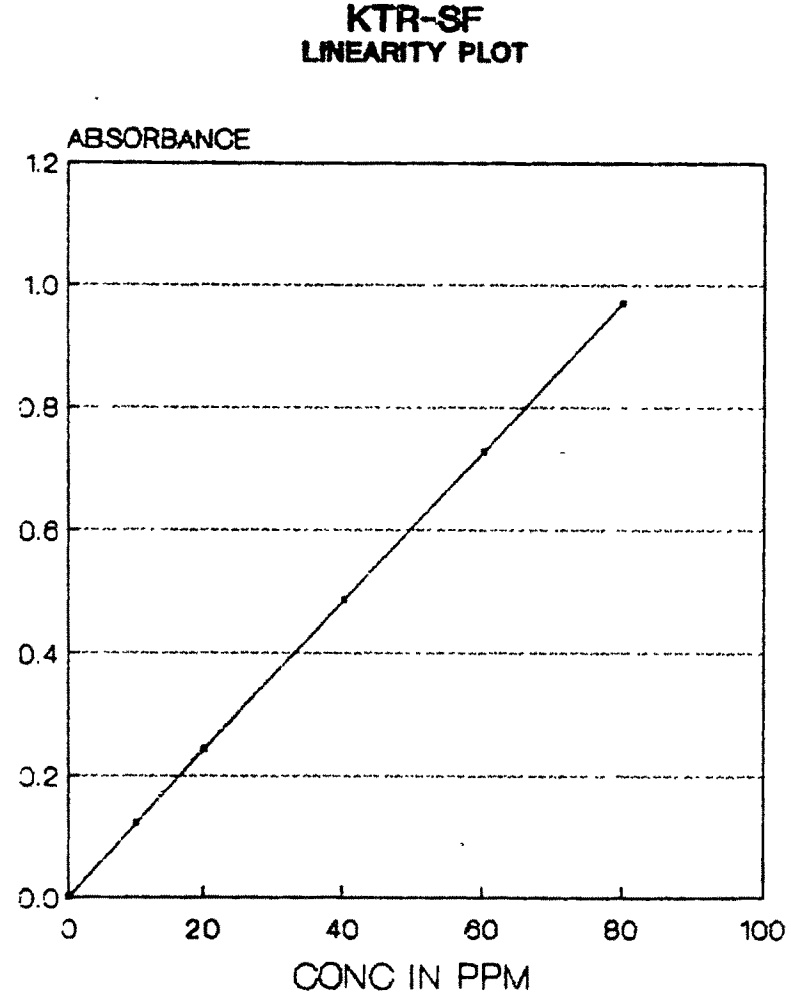


Fig. 2.9b : Linearity plot of KTR-SF method

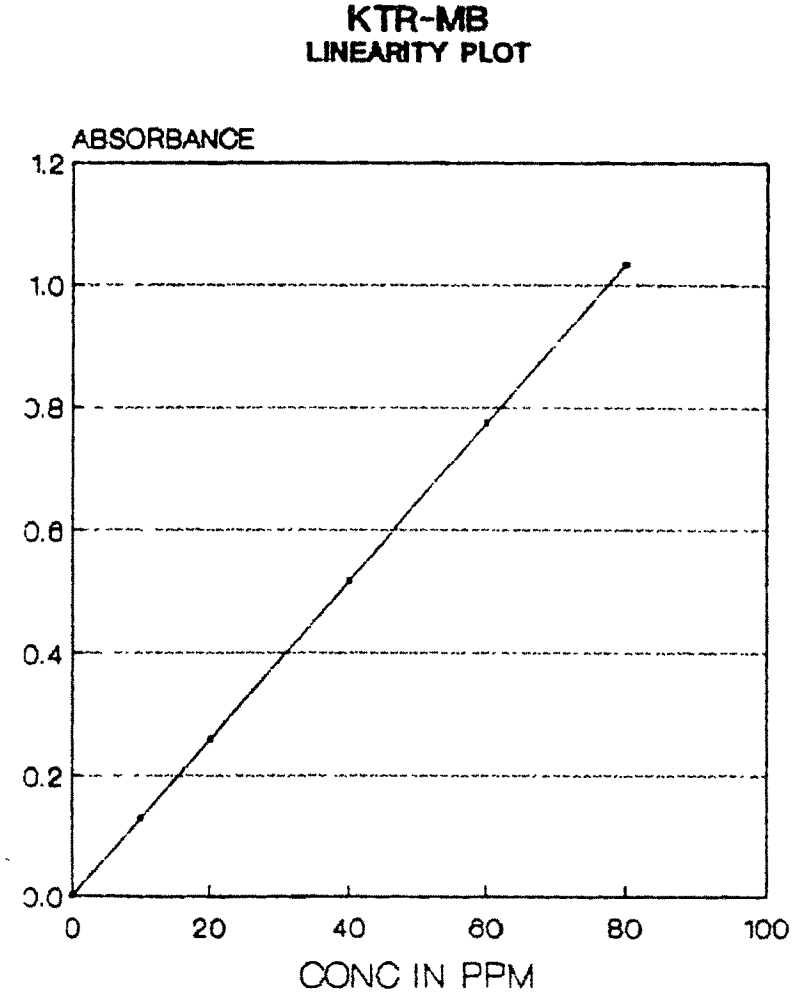


Fig. 2.9c : Linearity plot of KTR-MB method

Table 2.17: Results of KTR determination in tablets and injectables.

Dosage form	Label claim (mg)	% Found (± SD)		
		MB	SF	HPLC*
Tablet	10	98.27	98.25	98.21
		(0.60)	(0.56)	(0.52)
Injectable	15 (per ml)	100.12	100.19	100.14
		(0.56)	(0.48)	(0.73)
Injectable	30 (per ml)	99.49	99.51	99.57
		(0.54)	(0.52)	(0.64)

* Ref. 56

Various parameters like pH of the buffer, concentration of the dye, volume ratio of solvents and the solvent for extraction were fixed after several experiments. It was found that 5 ml of dye solution was optimum in the drug concentration range studied. Among various solvents used for extraction chloroform gave satisfactory results. The colour remained stable for 20 min and intensity of colour decreased gradually thereafter.

The molar ratio of drug to reagent in the complex as determined by continuous variation method[82] was 1 : 1 in both the cases.

To establish the practicality of the method, marketed products of KTR (tablets and injectables) were analysed by the proposed methods and also by a reported HPLC method [56]. The results given in Table 2.17 shows that the proposed methods compare well with HPLC method in accuracy. The recovery of the drug was in the range of 98.52 to 100.26% confirms the non-interference of common excipients

used in tablet and injectable preparations. The proposed methods can be used as general methods for the spectrophotometric determination of KTR.

2.6 METHODS BASED ON CHARGE TRANSFER COMPLEXATION REACTIONS

DFS forms charge transfer complexes with reagents, viz 2,3,-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), 2,4,-Dichloro-6-nitrophenol (DCNP) and iodine in non-aqueous solvent. Spectrophotometric methods based on the reactions were developed for the quantification of DFS. The absorbance of coloured products were measured at 460 nm for DDQ, 425 nm for DCNP and 360 nm for iodine. FMD has been analysed by charge transfer complexation using the reagents viz. chloranil, DDQ, DCNP and iodine. KTR has been determined by charge transfer complexation with DDQ and DCNP.

2.6A.1 PROCEDURES

Method A-13 for DFS: Different aliquots of methanolic solution of DFS (400 $\mu g.ml^{-1}$, 0.5-3.0 ml) were transferred into 10 ml volumetric flasks and one ml of DDQ solution was added into each flask. The volume was made upto 10 ml with methanol and allowed to stand at 20-25°C for 5 min. The absorbance was measured at 460 nm against a reagent blank.

Method A-14 for DFS: Aliquots of methanolic solution of DFS (100 $\mu g.ml^{-1}$, 1-5 ml) were placed into a series of 10 ml volumetric flasks and one ml of DCNP solution was added. The volume was made upto 10 ml with methanol and allowed to stand at 20-25°C for 30 min. The absorbance was measured at 425 nm against a reagent blank.

Method A-15 for DFS: Aliquots of the drug solution in chloroform (200 $\mu g.ml^{-1}$, 0.2-1.0 ml) were transferred into 10 ml volumetric flasks and one ml of iodine solution was added. The flasks were allowed to stand at 20-25°C for 15 min. The volume was made upto 10 ml with chloroform and the absorbance was measured at 360 nm against a reagent blank.

2.6A.2 RESULTS AND DISCUSSION

Methods A-13 to A-15: Diclofenac sodium was found to yield intense colours with the reagents DDQ and iodine in methanol and chloroform due to the formation of charge transfer complexes[83]. Similarly the DFS was found to react with the reagent DCNP in methanol to yeild an intense colour. An electronic absorption additional to the absorption of the components was observed. This may be ascribed to an intermolecular charge transfer from the donor to the acceptor. Due to the presence of a nitro and two chloro substituents, DCNP is expected to act as a π-acceptor. This is supported by the fact that no such CT band is observed in this region when the nitro group in DCNP is replaced by an amino group.

Optimization of parameters: Several experiments were conducted to optimize the conditions for maximum colour development. Among the various solvents tried as the reaction medium, methanol gave satisfactory and more stable colour for DDQ and DCNP methods whereas chloroform gave better results for iodine method. One ml of the three reagents of stated concentration was required to get maximum colour with an optimum time of 5 min for DDQ, 30 min for DCNP and 15 min for iodine. The colour remained stable for 1 hour in all the cases studied.

Fig. 2.10 shows the absorption apectra of the coloured products from these reactions with λmax at 396 nm and 460 nm for DDQ, 425 nm for DCNP and 360

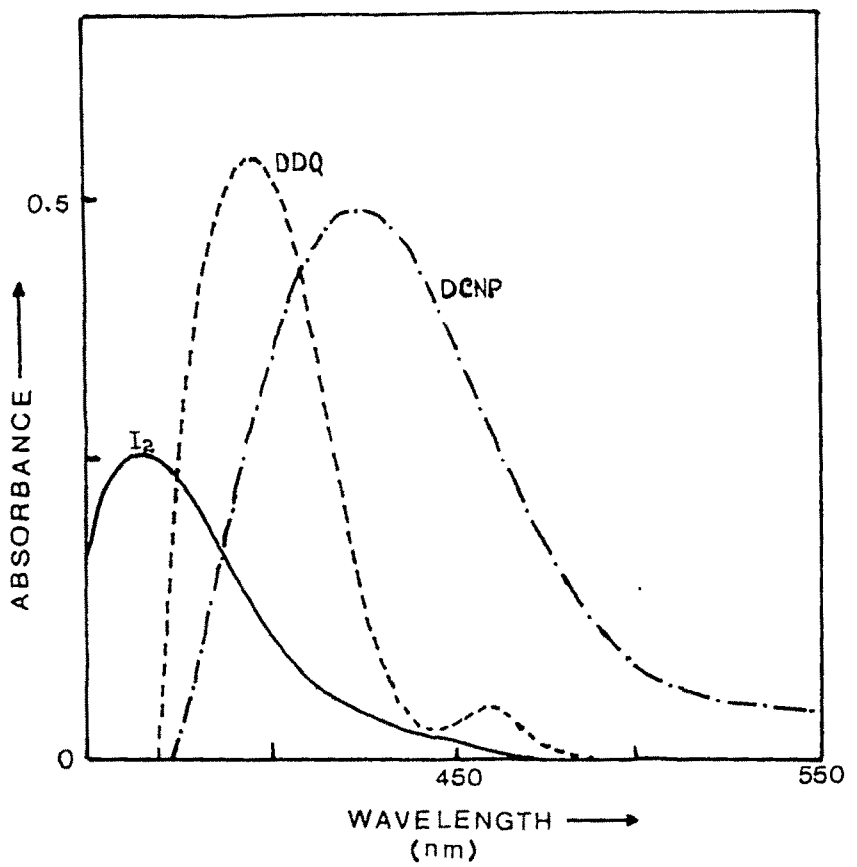


Fig. 2.10a : Absorption spectra of reaction products of DFS with DDQ, DCNP, Iodine

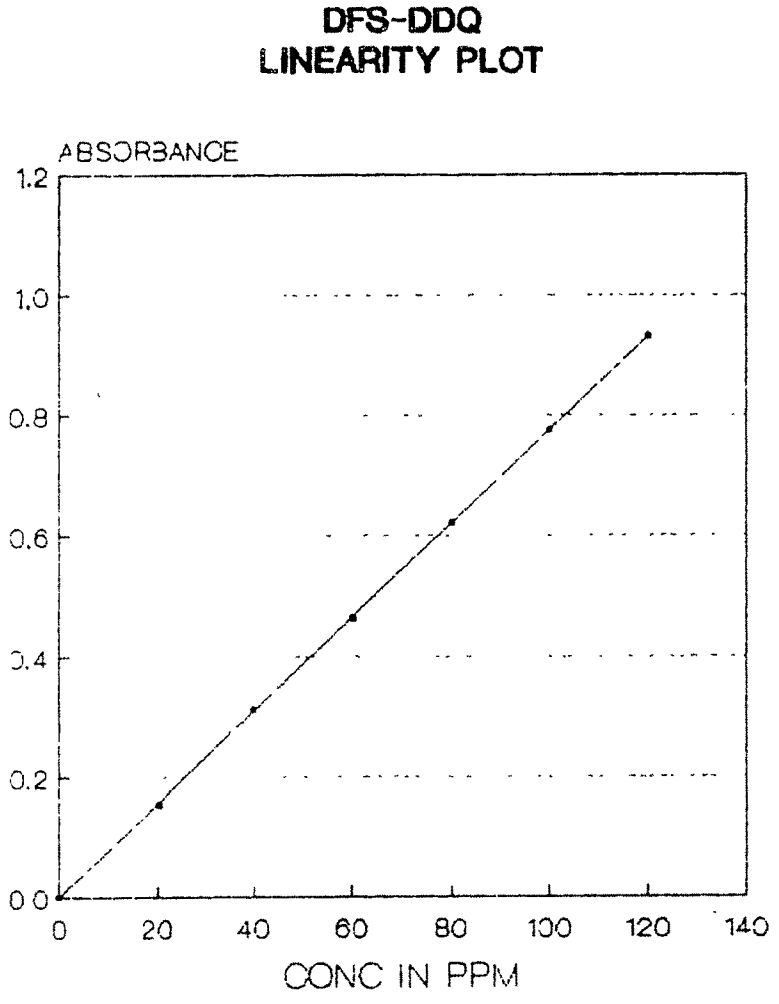


Fig. 2.10b : Linearity plot of DFS-DDQ method

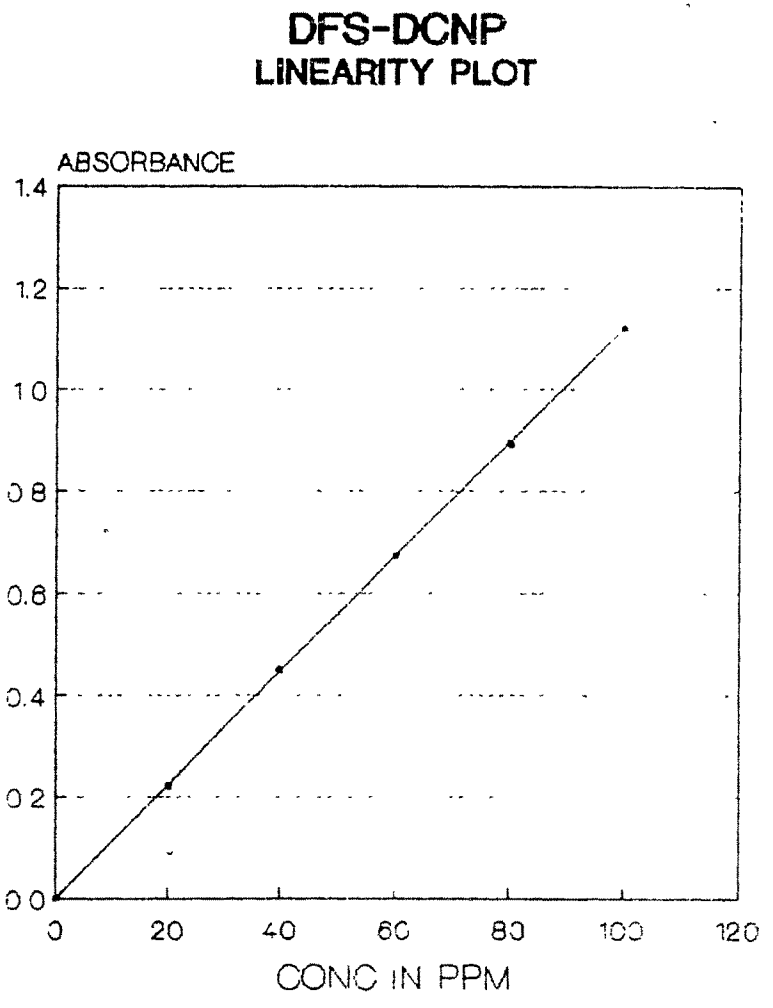


Fig. 2.10c : Linearity plot of DFS-DCNP method

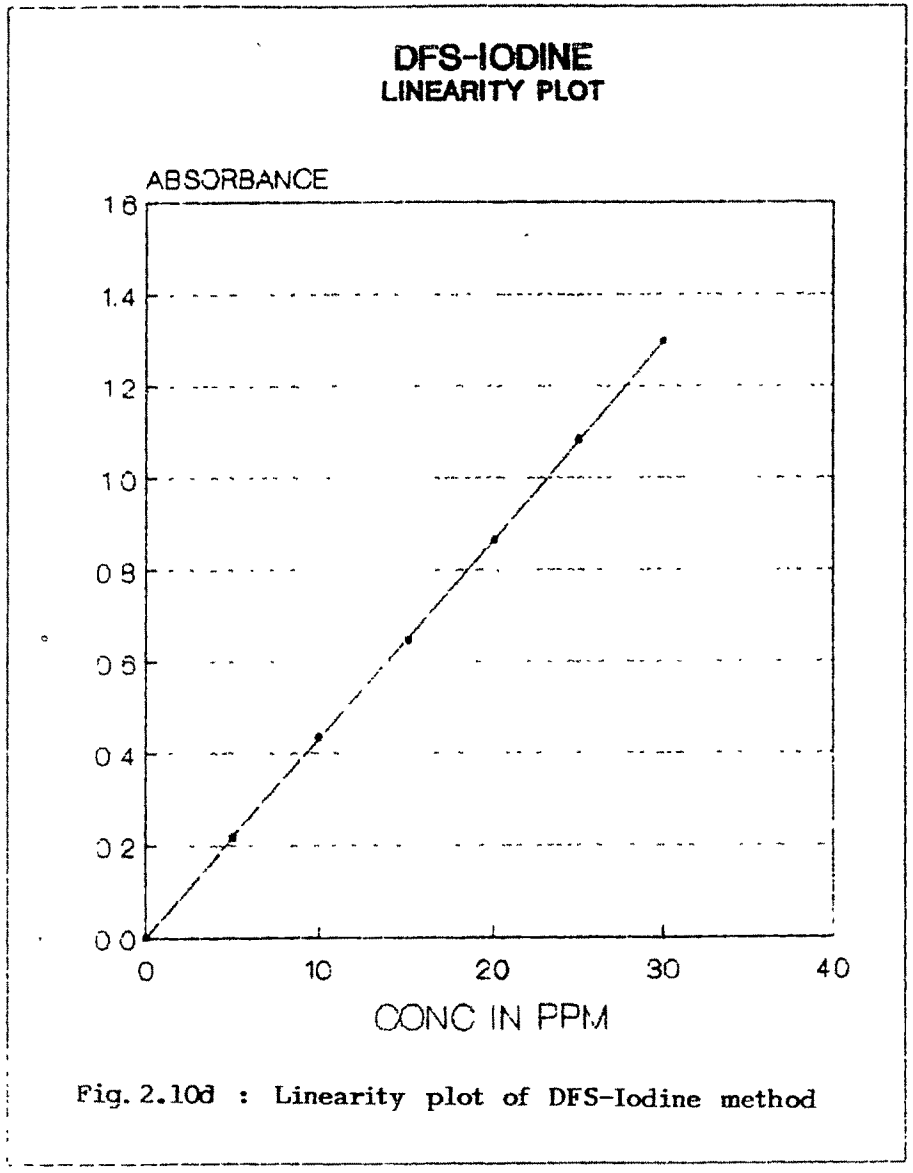


Fig. 2.10d : Linearity plot of DFS-Iodine method

nm for iodine in the range studied. Absorbance measurements at these wavelengths form the basis for quantitation of DFS. In the case of complex with DDQ, the sensitivity can be increased by measurements of absorption at 396 nm. Spectral data obtained from the calibration experiments are given in Table 2.18.

Table 2.18: Spectral data for the reaction products of DFS with DDQ, DCNP and iodine.

Reagent	λmax nm	Linearity range $\mu g.ml^{-1}$	Molar absorptivity $L.mol^{-1}.cm^{-1}$	Intercept coefficient	Correlation
DDQ	460	15-120	$2.30x10^3$	0.0680	0.9998
DCNP	425	10-100	$3.48x10^3$	0.0505	0.9999
Iodine	360	2-20	$1.77x10^4$	0.0387	0.9997

The molar ratio of the drug to reagent in the complexes as determined by continuous variation method[82] was 1:1 in all the three cases. To establish the practicality of the methods marketed products of DFS (tablets and injectables) were analysed by the proposed methods and also by a reported HPLC method[30]. The results given in Table 2.19 show that the proposed methods compare well with the HPLC method in accuracy although they are simple and inexpensive.

For recovery studies known amount of standard DFS was added to the sample solutions which had been analysed earlier. The recovery of the drug was 98.25 - 100.82%. Common excipients found in the tablet and injectable preparations did not interfere. However, interference was observed in formulations containing paracetamol in combination with the drug. In such cases, DFS was extracted from acidic solution with chloroform. After evaporation of chloroform the residue was dissolved in methanol for methods using DDQ and DCNP.

Table 2.19 : Determination of DFS in tablets and injectables by charge transfer complexation.

Dosage form	Label claim mg	% Found (± SD)**			
		DDQ	DCNP	Iodine	HPLC
Tablet*	25	98.24 (0.52)	97.92 (0.42)	98.12 (0.76)	99.12 (0.84)
Tablet*	50	99.12 (0.62)	99.25 (0.36)	99.18 (0.30)	100.22 (0.96)
Injectable*	25	100.21 (0.72)	100.35 (0.58)	100.18 (0.92)	100.56 (0.73)

** Average of three determinations.

* Products of CIBA-GIEGY, Germany

2.6B.1 PROCEDURES

Method C-5 for FMD: Aliquots of methanolic solution of FMD (1 mg.ml^{-1}) were transferred into a series of 10 ml volumetric flasks and 2 ml of chloranil reagent was added. The volume was made upto 10 ml with methanol and allowed to stand at 28-30°C for 30 min. The absorbance was measured at 458 nm against a reagent blank.

Method C-6 for FMD: Aliquots of methanolic solution of FMD (1 mg.ml^{-1}, 0.5-4.0 ml) were placed in a series of 10 ml volumetric flasks and 2 ml of DDQ reagent was added. The volume was made upto 10 ml with methanol and allowed to stand at 28-30°C for 20 min. The absorbance was measured at 460 nm against a reagent blank.

Method C-7 for FMD: Aliqouts of methanolic solutions of FMD (200 $\mu g.ml^{-1}$, 1-5 ml) were transferred into a series of 10 ml volumetric flasks and 1 ml of DCNP solution was added to each flask. The volume was made upto 10 ml with methanol and allowed to stand at 28-30°C for 30 min. The absorbance was measured at 425 nm against a reagent blank.

Method C-8 for FMD: Aliqouts of standard drug solution (0.5 $mg.ml^{-1}$, 0.1-0.5 ml) were transferred into a series of 10 ml volumetric flasks. The volume was adjusted to 0.5 ml in all the flasks using methanol and one ml of iodine solution was added. The solutions were allowed to stand for 15 min at 20-25°C. The volume was made upto 10 ml with chloroform and the absorbance was measured at 360 nm against a reagent blank.

For tablet analysis weight of tablet powder equivalent to 20 mg of the drug was stirred with 30 ml of methanol and diluted to 50 ml with the same solvent. The solution was filtered and 5 ml of the filtrate was used for the method using chloranil and DDQ. In the case of the method using DCNP, 5 ml of the filtrate was further diluted to 50 ml with methanol and 5 ml of the resultant solution was used for colour development. In the method using iodine, weight of tablet powder equivalent to 25 mg of drug was dissolved in 100 ml methanol. The solution was filtered and 0.5 ml of the filtrate was used for colour development.

2.6B.2 RESULTS AND DISCUSSION

Methods C-5 to C-8: Famotidine was found to yield intense colours with chloranil, DDQ and DCNP in methanol; most probably due to the formation of charge-transfer complexes[83], because the reagents are known π-acceptors. Fig. 2.11 shows the absorption spectra of coloured products formed with wavelength

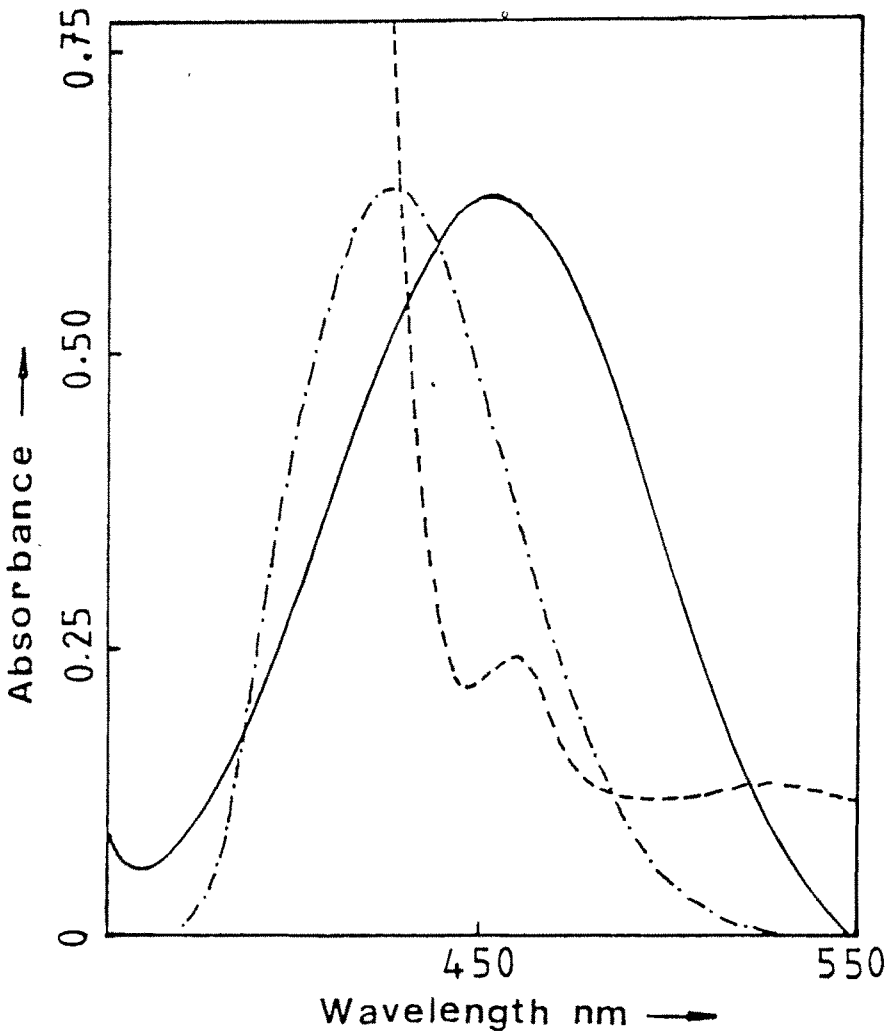


Fig. 2.11a : Absorption spectra of Famotidine-Chloranil (————), -DDQ (– – – – –) and -DCNP (–·–·–·–)

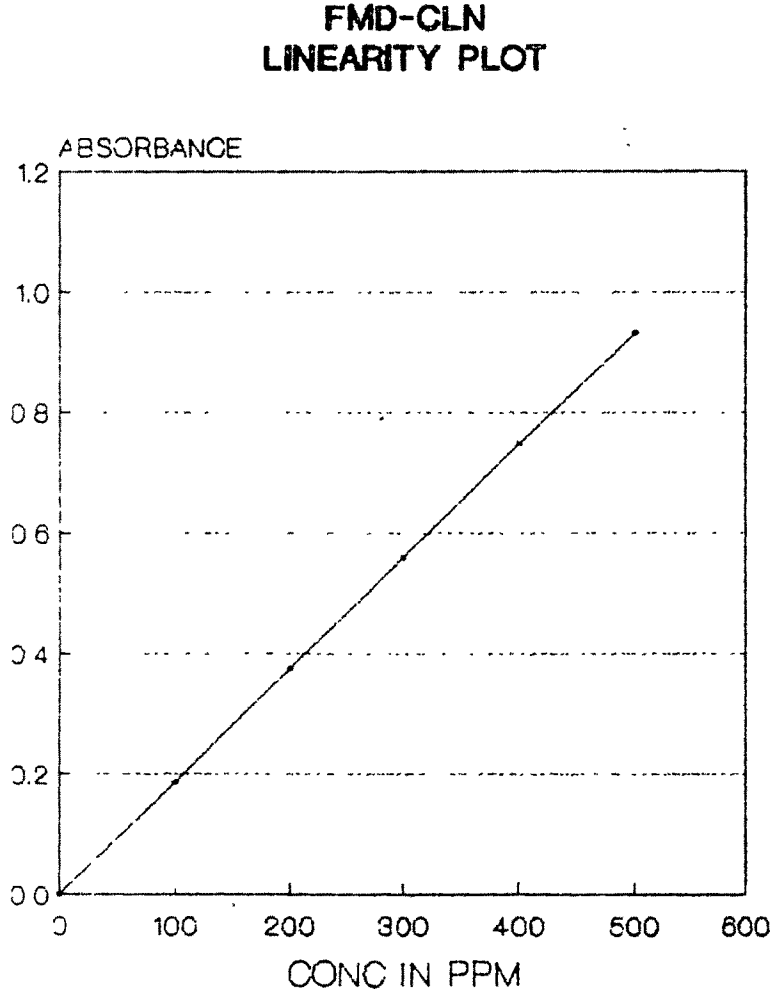


Fig. 2.11b : Linearity plot of FMD-CLN method

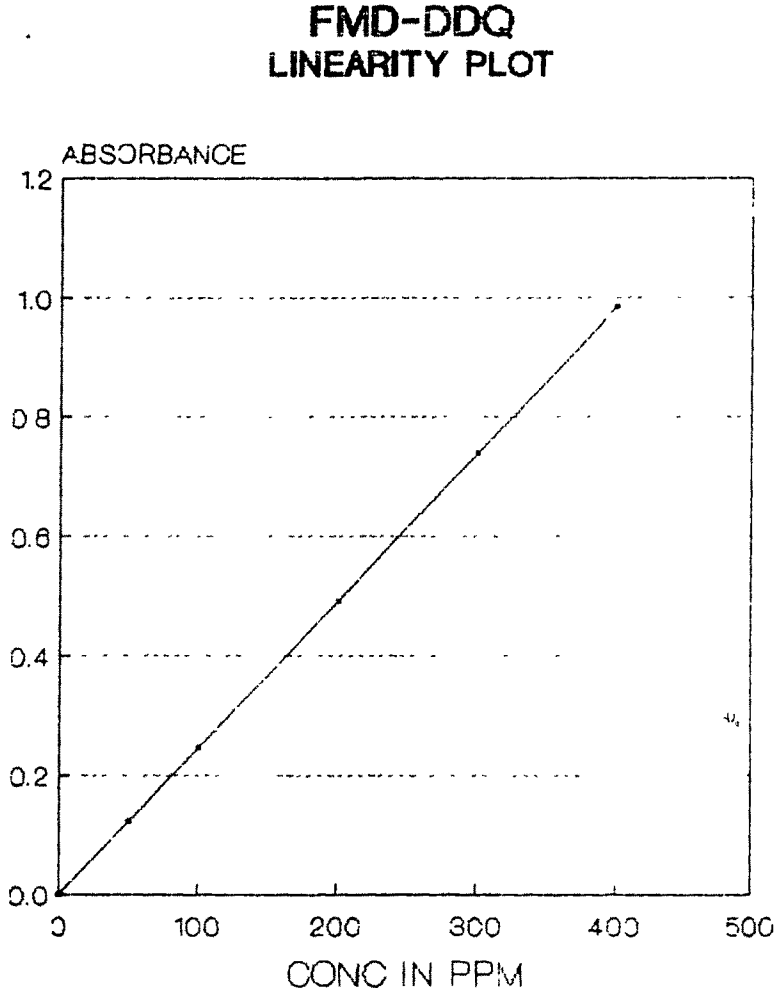


Fig. 2.11c : Linearity plot of FMD-DDQ method

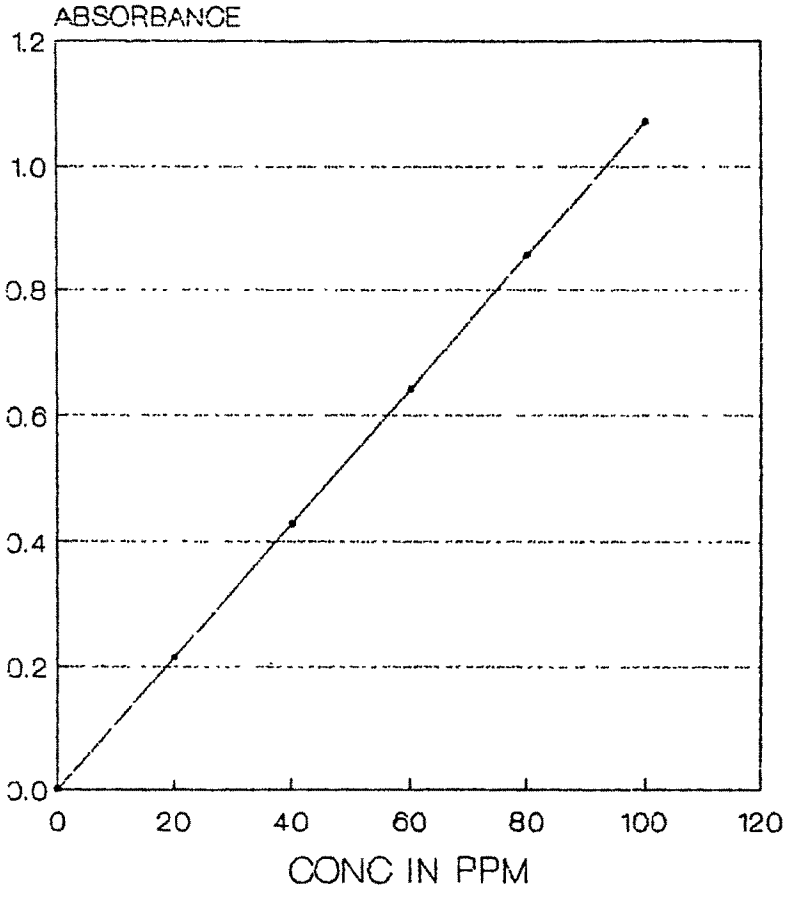


Fig. 2.11d : Linearity plot of FMD-DCNP method

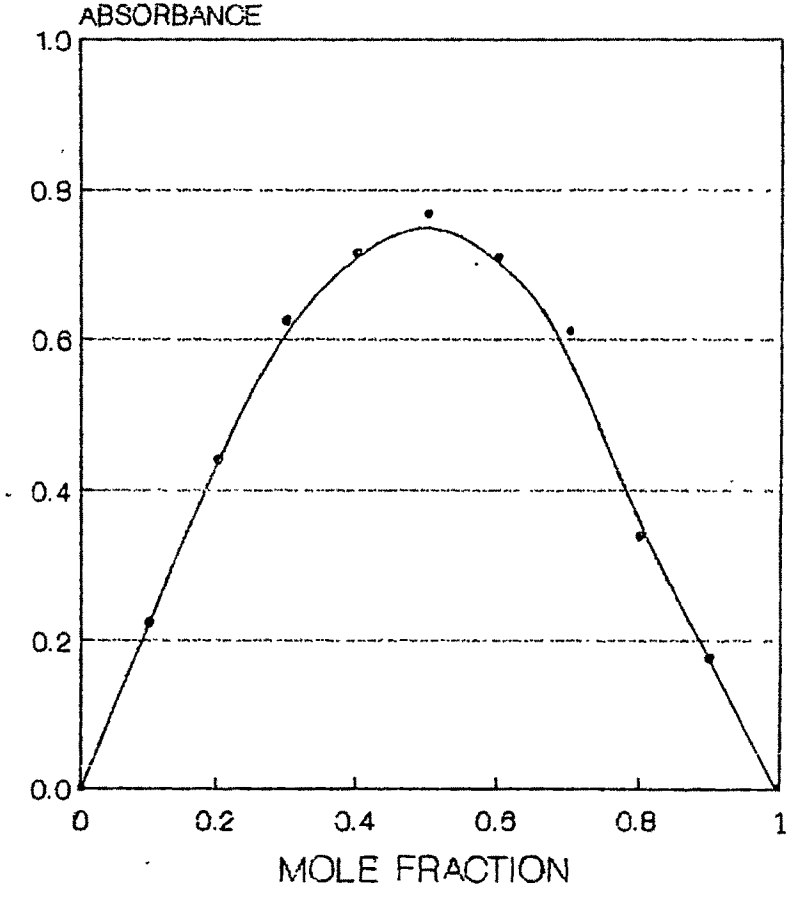


Fig. 2.11e : Mole ratio plot of FMD-DCNP complex

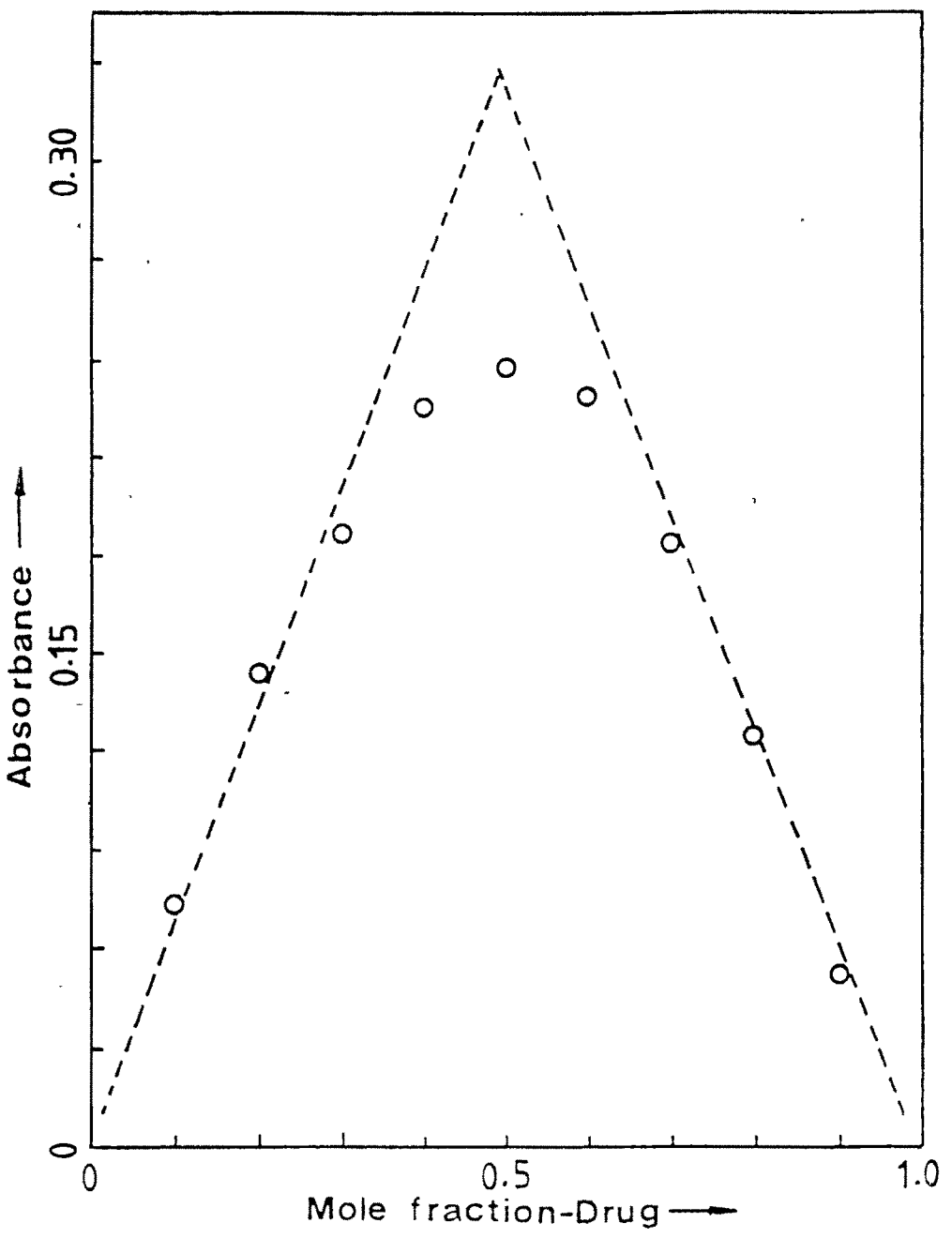


Fig. 2.11f : Continuous variation plot of FMD-CLN complex

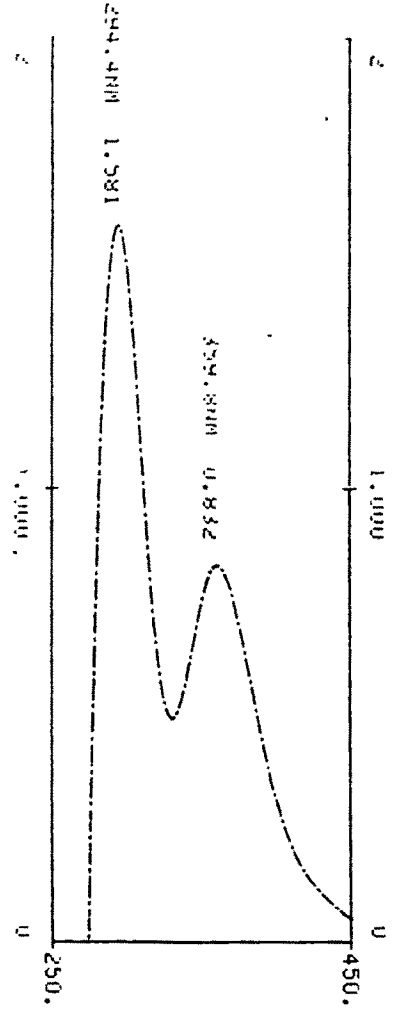


Fig. 2.11g : Absorption spectra of FMD-Iodine Complex

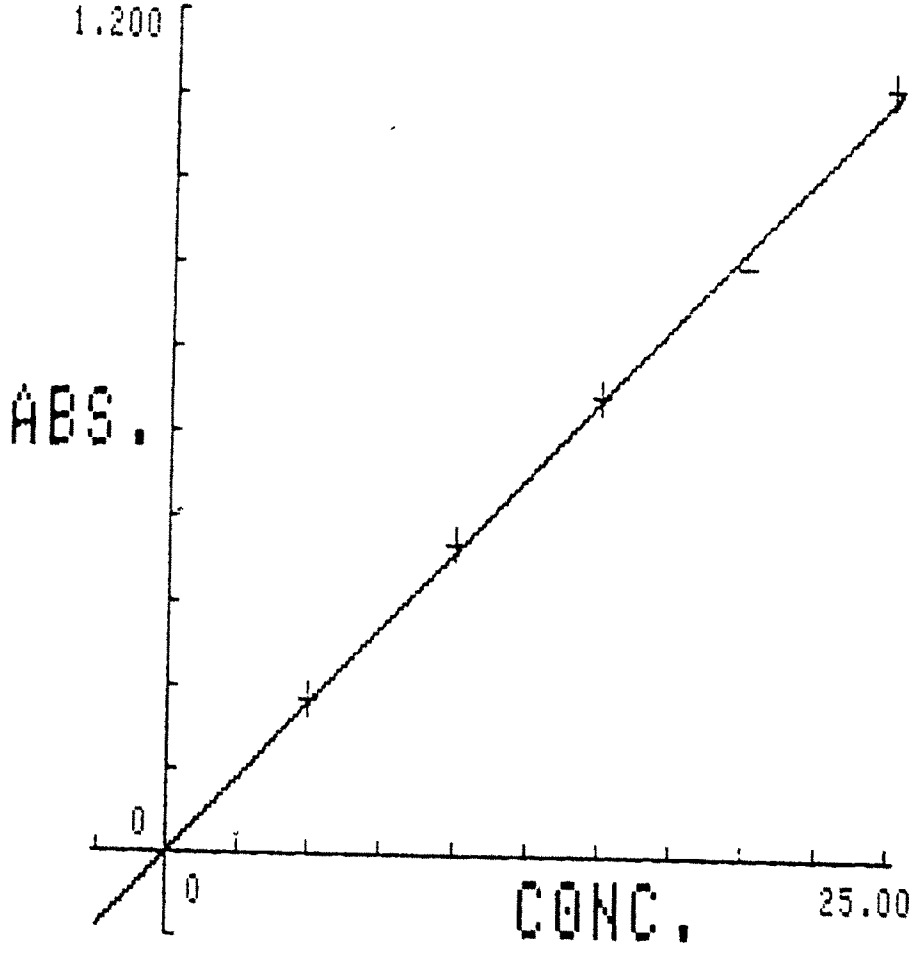


Fig. 2.11h : Linearity plot of FMD-Iodine method

of maximum absorption (λmax) at 458 nm for chloranil, 392 nm and 460 nm for DDQ and 425 nm for DCNP in the range studied. In the case of method using iodine, the resultant product of the drug with iodine was lemon yellow in colour when chloroform was used as the solvent. The change in violet colour of iodine solution is due to the formation of an intermolecular charge transfer complex involving an electron transfer from donor (FMD) to acceptor (iodine). The absorption spectra of reaction products (Fig. 2.11) show the characteristic n-donor iodine charge transfer complexes with λ max at 295 nm and 360 nm. Since the drug itself has an absorption maximum near 295 nm, the second wavelength was chosen for absorption measurements. Absorbance measurements were made at above wavelengths to construct the calibration graph and subsequently for the analysis. Spectral data for the reaction products of FMD with the four reagents are given in Table 2.20.

Table 2.20 : Spectral data for the reaction products of FMD with chloranil, DDQ, DCNP and iodine.

Reagent	λmax nm	Linearity range $\mu g.ml^{-1}$	Molar absorptivity $L.mol^{-1}.cm^{-1}$	Intercept	Correlation coefficient
Chloranil	458	50-500	$0.67x10^3$	0.0345	0.9995
DDQ	460	40-400	$0.82x10^3$	0.0457	0.9997
DCNP	425	10-100	$3.67x10^3$	0.0326	0.9998
Iodine	360	1.6-16	$1.41x10^4$	0.0464	0.9998

The relative sensitivity of the four reagents in analytical work can be compared by the apparent molar absorptivity values of the chromogens (Table 2.20). In the

case of DDQ the sensitivity of the method can be increased several folds by measurement at 392 nm.

The molar ratio of the drug to reagent established by the application of Job's continuous variation method[82] was found to be 1:1 in all the cases except in the case of iodine, where the ratio was found to be 1:2. Fig 2.11 shows the results obtained for FMD with chloranil.

Optimization of parameters

Several experiments were conducted to fix the optimum parameters, viz concentration of the reagent, reaction time and solvent for the reaction. It was established that 2 ml of chloranil or DDQ reagent was required for maximum colour development whereas for DCNP and iodine method one ml was found to be optimum. The time required for maximum colour development was 30 min with DDQ, DCNP and chloranil and 15 min with iodine. The colour remains stable for one hour in all the cases. Among the various solvents used as the reaction medium, methanol gave satisfactory and more stable colour with DDQ, DCNP and chloranil. However, in the case of iodine, chloroform medium gave more stable colour. Since FMD is slightly soluble in chlorform and the presence of high amount of methanol will impart yellow colour to the reaction mixture, the volume of methanol was restricted to a minimum. Also, it was observed that low quantity of methanol used in the study has not affected the reagent blank absorbance.

The practicality and suitability of the proposed methods for quantitation of FMD can be judged by the results of tablet analysis given in Table 2.21.

For recovery study known amount of FMD was added to tablet solutions which had been analysed earlier. The recovery of the drug was 98.87-100.72% which

showed the absence of interference form common excipients like talc, magnesium stearate, silicon dioxide, starch, lactose, acacia and gelatin used in tablet formulation. The results obtained by the proposed methods are comparable with those of the USP method.

Table 2.21 : Results of famotidine tablet analysis by charge transfer compl exation.

Tablet	Label claim (mg)	% Found (± SD)				
		Chloranil method	DDQ method	DCNP method	Iodine method	USP-1990 method
LotA	20	99.70 (0.68)	99.68 (0.86)	99.64 (0.73)	99.65 (0.92)	99.64 (0.75)
Lot B	20	98.77 (0.83)	98.81 (0.75)	98.90 (0.68)	98.96 (0.86)	98.86 (1.06)
Lot C	40	100.13 (0.90)	100.06 (0.96)	100.09 (0.56)	100.14 (0.88)	100.07 (0.87)
Lot D	40	99.05 (0.74)	99.12 (0.64)	99.18 (0.42)	99.17 (1.04)	99.10 (0.58)

2.6C.1 PROCEDURES

Method D-3 for KTR: Aliqouts of the KTR solution in methanol (200 $\mu g.ml^{-1}$, 0.5-4.0 ml) were transferred into 10 ml volumetric flasks and 2 ml of 0.2% of DDQ solution were added into each flask. The volume was made upto 10 ml with methanol and allowed to stand at 20-25°C for 30 min. The absorbance was measured at 392 nm against a reagent blank.

Method D-4 for KTR: Aliquots of the KTR solution in methanol (200 $\mu g.ml^{-1}$ 0.5-4.0 ml) were transferred into 10 ml volumetric flasks and one ml of DCNP solution was added into each flask. The volume was made upto 10 ml with methanol and

allowed to stand at 20-25°C for 30 min. The absorbance was measured at 425 nm against a reagent blank.

2.6C.2 RESULTS AND DISCUSSION

Methods D-3 and D-4: KTR was found to yield intense colours with DDQ and DCNP in methanol, due to the formation of charge transfer complexes, because these reagents are known π-acceptors. Fig.2.12 shows the absorption spectra of the coloured products formed with wavelength of maximum absorption at 392 nm for DDQ and 425 nm for DCNP. Spectral data for the reaction products of KTR with both the reagents are given in Table 2.22.

Table 2.22: Spectral data for the reaction products of KTR with DDQ and DCNP.

Reagent	λmax nm	Linearity range $\mu g.ml^{-1}$	Molar absorptivity $L.mol^{-1}.cm^{-1}$	Intercept	Correlation coefficient
DDQ	392	10-80	$0.414x10^4$	0.0599	0.9997
DCNP	425	10-80	$0.420x10^4$	0.0046	0.9998

Several experiments were conducted to optimize the conditions for maximum colour development. Among the various solvents used as the reaction medium, methanol gave satisfactory and more stable colour. One ml of DCNP and 2 ml of DDQ reagents were required to get maximum colour with optimum time of 30 min. The colour remained stable for one hour in both the cases studied.

The molar ratio of the drug to reagent in the complexes as determined by continuous variation method[82] was 1:1 in both the cases.

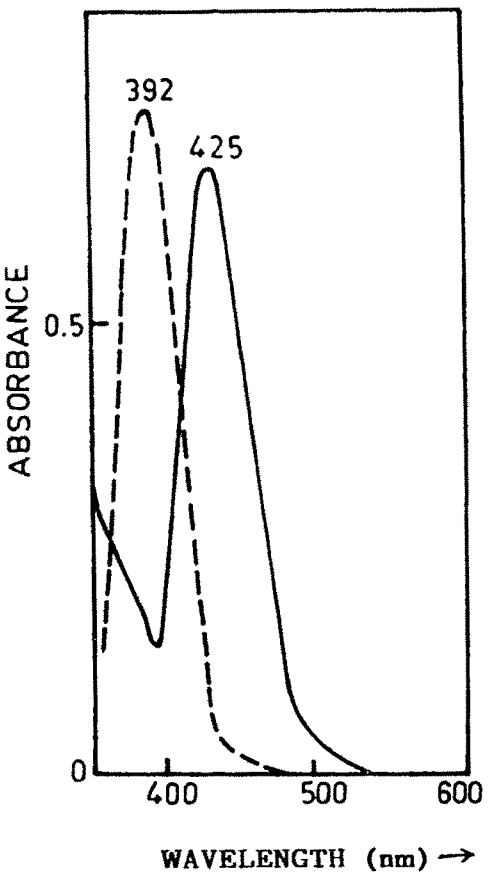


Fig. 2.12a : Absorption spectra of charge transfer complexes of KTR with DDQ and DCNP

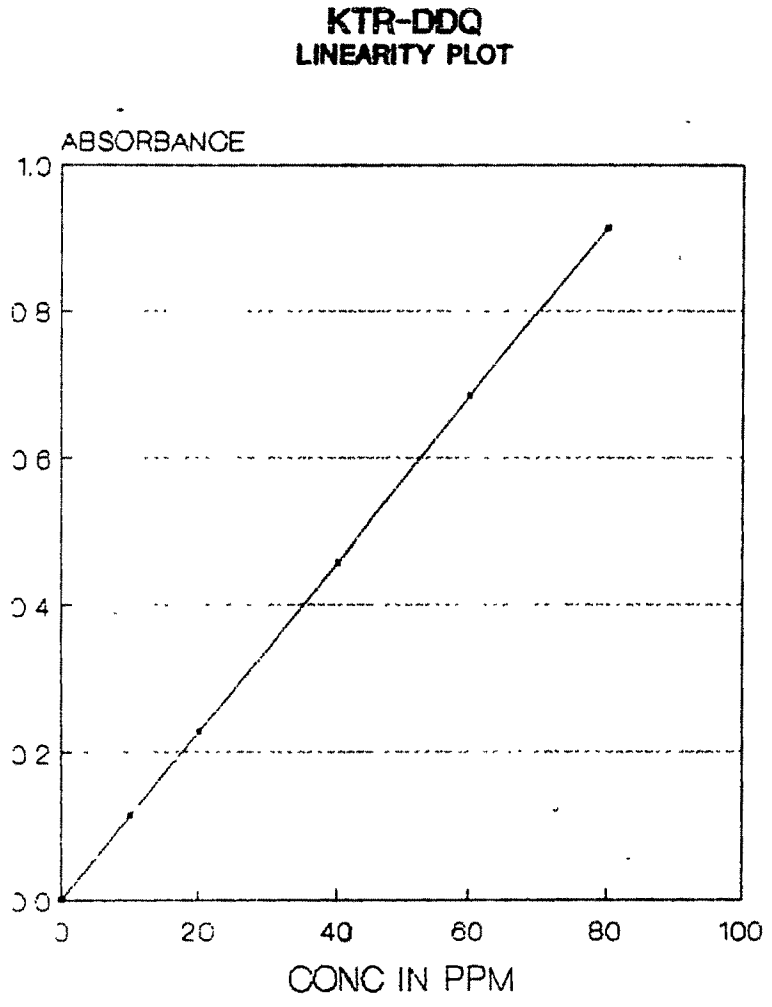


Fig. 2.12b : Linearity plot of KTR-DDQ method

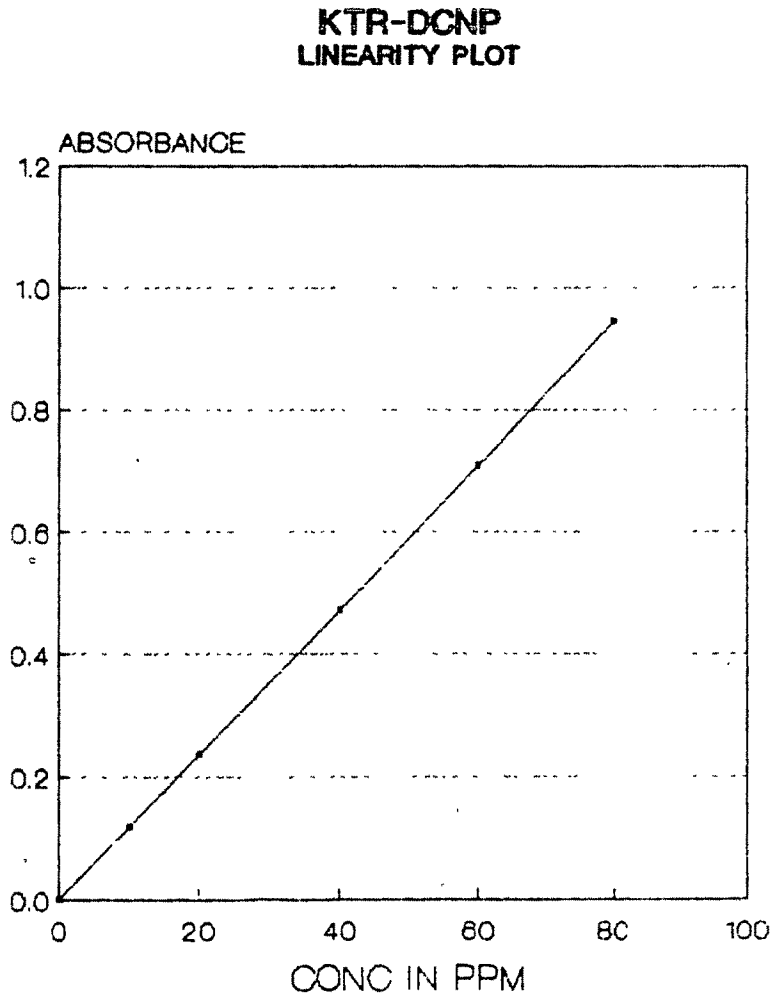


Fig. 2.12c : Linearity plot of KTR-DCNP method

Marketed products of KTR were analysed by the proposed methods and also by a reported HPLC method[56]. The results given in Table 2.23 show that the proposed methods are comparable with HPLC method in accuracy.

Table 2.23: Determination of KTR in tablets and injectable using DDQ and DCNP.

Dosage form	Label claim mg	% Found (± SD)		
		DDQ	DCNP	HPLC
Tablet	10	98.26 (0.42)	98.26 (0.56)	98.24 (0.52)
Injectable	15(per ml)	100.18 (0.38)	100.27 (0.34)	100.14 (0.73)
Injectable	30(per ml)	99.56 (0.44)	99.61 (0.42)	99.57 (0.64)

In recovery studies, the recovery of the drug were found in the range of 98.52 to 100.26%. Common excipients like starch, talc, silicon dioxide, lactose, magnesium stearate, gelatin, dibasic calcium phosphate, mannitol, methylparaben, and sodium metabisulphite used in tablet and injectable preparations did not interfere.

The suggested methods have the advantage of being simple, accurate and sensitive. The proposed methods utilize single solvent and single step reaction.

2.7 METHODS BASED ON COMPLEXATION WITH METAL IONS

DLZ forms coloured complexes with cobalt (II) thiocyanate and iron (III) thiocyanate in acidic media. The complexes formed are quantitatively extracted with benzene which showed λ max at 630 nm and 495 nm respectively. Another

simple but selective spectrophotometric method has been described for the determination of DLZ in tablets. The method is based on the reaction of the drug with alkaline hydroxylamine to produce hydroxamic acid, which in turn forms a purple coloured complex with ferric ion in acidic medium with λ max at 500 nm.

2.7.1 PROCEDURE

Method B-12 for DLZ: Aliqouts of aqueous solution of DLZ (0.2-2.0 ml, 3 $mg.ml^{-1}$) were placed in a series of 25 ml volumetric flasks. The total volume was adjusted to 2 ml in all the flasks with water. To each flask 0.5 ml of 2 M hydrochloric acid, 2 ml of cobalt thiocyanate solution and 10 ml benzene were added. The flasks were stoppered, shaken for 5 min and allowed to stand for clear separation of the two layers. The blue coloured benzene layer was transferred into a dry test tube containing about 500 mg of anhydrous sodium suphate. The absorbance was measured at 630 nm against a reagent blank.

Method B-13 for DLZ: Aliqouts of aqueous of DLZ (1 $mg.ml^{-1}$, 0.2-1.0 ml) were placed in a series of 25 ml volumetric flasks. The total volume was adjusted to one ml in all the flasks with water. To each flask 0.5 ml of ammonium thiocyanate solution and 0.5 ml of hydrochloric acid solution were added. Further, one ml of hydrochloric acid (5 M) and 10 ml of benzene were added and the flasks were shaken for 5 min. The flasks were allowed to stand for clear separation of the two layers. The red coloured benzene layer was transferred into a dry test tube containing about 500 mg of sodium sulphate and the absorbance was measured at 495 nm against a reagent blank.

Method B-14 for DLZ: Into a series of 25 ml volumetric flasks different volumes of methanolic solution of the drug (4 $mg.ml^{-1}$, 1-5ml) were pipetted out. The

total volume was adjusted to 5 ml in all the flasks with methanol. To each flask 3 ml of hydroxylamine reagent was added and the solutions were kept at 72 ± 0.5°C in a constant temperature water bath. After 20 min the solutions were cooled to room temperature. To each flask one ml of ferric reagent and 2 ml of dilute perchloric acid solutions were added with shaking. The volume was made upto the mark with methanol and the absorbance was measured within 5 min at 500 nm against a reagent blank.

Twenty tablets were weighed and powdered. A quantity of powdered tablets equivalent to 60 mg of drug was extracted with 60 ml chloroform in two or three instalments. The filter was washed with 10 ml chloroform. The combined filterate was evaporated to dryness and the residue was dissolved in 50 ml of methanol. A 5 ml volume of the filtrate was used for colour development by the method described above. Amount of the drug corresponding to the absorbance value was found from the calibration graph and the content of diltiazem hydrochloride per tablet was calculated using the dilution factor.

2.7.2 RESULTS AND DISCUSSION

Method B-12 and B-13: Diltiazem hydrochloride was found to react with cobalt(II) thiocyanate and iron(III) thiocyanate in acidic media at room temperature and the complex formed in both the cases was extracted with benzene. Fig. 2.13 shows the absorption spectra of coloured complexes with wavelength of maximum absorption. Spectral data for the coloured complex with both the reagents are given in Table-2.24.

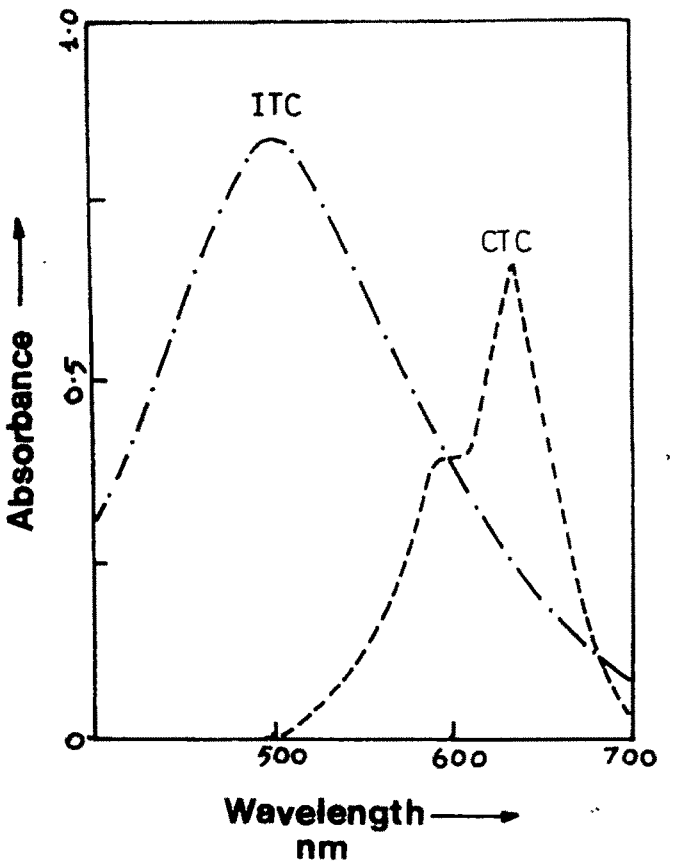


Fig. 2.13a : Absorption spectra of coloured complexes of DLZ with CTC and ITC

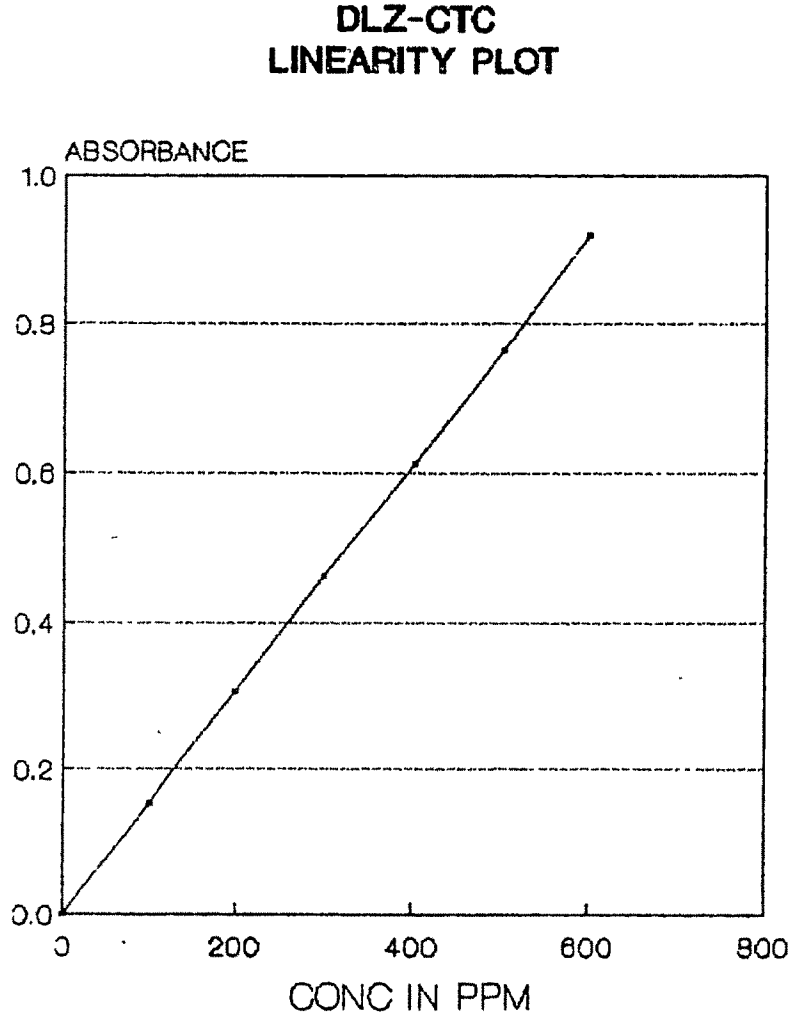


Fig. 2.13b : Linearity plot of DLZ-CTC method

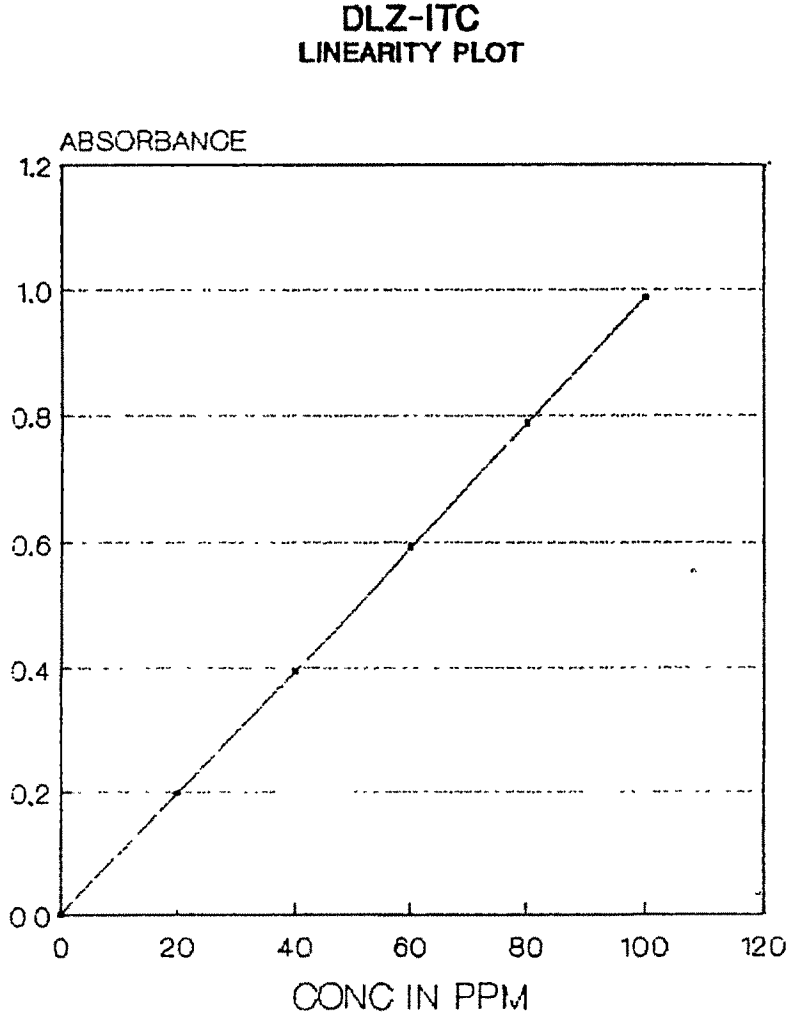


Fig. 2.13c : Linearity plot of DLZ-ITC method

Table 2.24: Spectral data for coloured complexes of DLZ with CTC and ITC.

Reagent	λmax nm	Linearity range $\mu g.ml^{-1}$	Molar absorptivity $L.mol^{-1}.cm^{-1}$	Intercept	Correlation coefficient
CTC	630	60-600	$0.721x10^3$	0.0723	0.9998
I T C	495	10-100	$0.581x10^4$	0.0847	0.9997

The molar ratio of the drug to reagent was established by Job's method[82]. The concentration of the drug and reagents were both 0.04 M. The plots show the formation of 1 : 1 complex of the drug with cobalt (II) and iron (III) ions. The practicality and suitability of these methods for quantification of DLZ can be judged by the results of tablet analysis given in Table 2.25.

Table 2.25 : Results fo DLZ tablet analysis using CTC and ITC.

Tablet	Label claim mg	% Found (± SD)		
		CTC method	ITC method	USP method
Lot A	30	97.42 (0.76)	97.38(0.58)	97.34(0.36)
Lot B	30	99.52 (0.65)	99.45(0.60)	99.40(0.28)
Lot C	60	98.12 (0.34)	98.16(0.45)	98.14(0.34)
Lot D	60	98.36 (0.72)	98.32(0.52)	98.34(0.40)

The recovery of the drug was in the range 98.22-100.48%. The results obtained by the proposed methods are comparable with those of the USP method[10]. However, interference was observed if desacetyl diltiazem hydrochloride is present in the formulation.

Method B-14: An organic ester is known to react with hydroxylamine in alkaline medium[84-87] to form a hydroxamic acid, which combines easily with ferric ions under suitable conditions to form a characteristic red to purple chelate: (Scheme 2).

$$CH_3\text{-}CO\text{-}R + NH_2OH.HCl + NaOH \longrightarrow CH_3\text{-}CO\text{-}NHOH + ROH + NaCl + H_2O.$$

$$CH_3\text{-}CO\text{-}NHOH + FeCl_3 \longrightarrow Fe(CH_3\text{-}CO\text{-}NHO)^{2+} + Cl^- + H^+$$

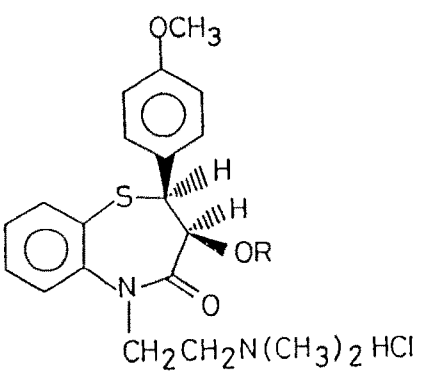
$$\rightleftharpoons Fe(CH_3\text{-}CO\text{-}NHO)_2^+$$

$$\rightleftharpoons Fe(CH_3\text{-}CO\text{-}NHO)_3$$

Diltiazem hydrochloride (**Ia**) contains an acetate moiety and therefore was found to undergo similar reactions to furnish a purple coloured ferric hydroxamate complex that can be measured spectrophotometrically. Since this reaction is specific for the acetate group, desacetyl diltiazem (**Ib**), if present in the formulaion is not likely to interfere.

Optimization of colour development conditions:

Temperature: The reaction of diltiazem with alkaline hydroxylamine was studied at 30, 50, 70, 80 and 90°C. It was found that maximum colour development occured at 70°C. Further experiments were performed in the temperature range of 70-72°C.



Ia R = $COCH_3$
Ib R = H

Reaction time: An investigation of the effect of reaction time showed that maximum colour was obtained when the reaction between hydroxylamine and diltiazem was carried out at 70-72°C for about 20 min. Also, the intensity of the colour rose to a maximum within 2 min after the addition of the ferric reagent and remained constant for another 3 min after which it gradually declined. Therefore, all absorbance measurements were made at about 4 min after the addition of the ferric reagent.

Solvent: The reaction was carried out using four solvents, namely methanol, ethanol, isopropanol and water. On the basis of solubility of the drug and optimal stability and intensity of colour, methanol solution of the drug was used for further experiments.

pH of the reaction: The effect of pH was investigated by using hydroxylamine reagent containing different amounts of sodium hydroxide. Experiments at pH 8.0, 10.0, 12.0, 13.0 and 13.8 showed that maximum colour intensity was obtained at pH 13.8. This may be ascribed to the increase in the rate of hydrolysis and also a shift in the ratio of the three coloured species as the pH was increased from 8.0 to 13.8 (Scheme 2).

The second variable in colour development was the amount of excess acid desirable over that necessary to neutralize the sodium hydroxide required for hydrolysis. An investigation of the effect of acid concentration showed that at lower acid concentration the colour was marginally more intense and the colour was more stable. A 2 ml volume of perchloric acid 14% (w/v) was found to be optimum for the purpose.

Under the optimized conditions the absorption spectrum of the complex showed a maximum at 500 nm (Fig. 2.14) with molar absorptivity 0.485×10^3 $L.mol^{-1}.cm^{-1}$ on the basis of the molecular weight of diltiazem hydrochloride. By following the procedure described above, absorbance measurements of the standard drug solution were made at 500 nm and a calibration curve was constructed by plotting absorbance versus concentration of the drug in $\mu g.ml^{-1}$. The curve was linear in the range of 50-800 $\mu g.ml^{-1}$. The intercept was 0.1390 with a correlation coefficient of 0.9993.

Analysis of tablets: Twenty tablets were weighed and powdered. A quantity of tablet powder equivalent to 60 mg of the drug was extracted with 60 ml of chloroform in two or three instalments. The filter was washed with 10 ml chloroform. The combined filtrate was evaporated to dryness and the residue was dissolved in 50 ml of methanol. A 5 ml volume of filtrate was used for the colour development by the described method. The amount of drug corresponding to the absorbance value was found from the calibration graph and the content of DLZ per tablet was calculated using the dilution factor. The results are given in Table 2.26.

Table 2.26: Results of diltiazem tablet analysis by ferric hydroxamate method.

Formulation	Labelled amount of diltiazem HCl (mg)	Amount found* (mg)	Recovery %
Tablet A	30	29.62	98.73
Tablet B	30	30.02	100.07
TabletC	60	58.72	97.87
TabletD	60	59.60	99.33

* Average of six determination

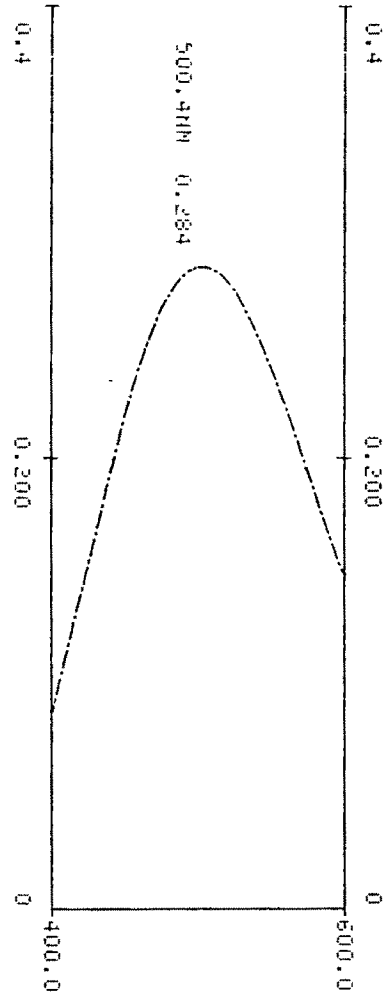


Fig. 2.14a : Absorption spectra of Complex formed between DLZ and Ferric hydroxamate

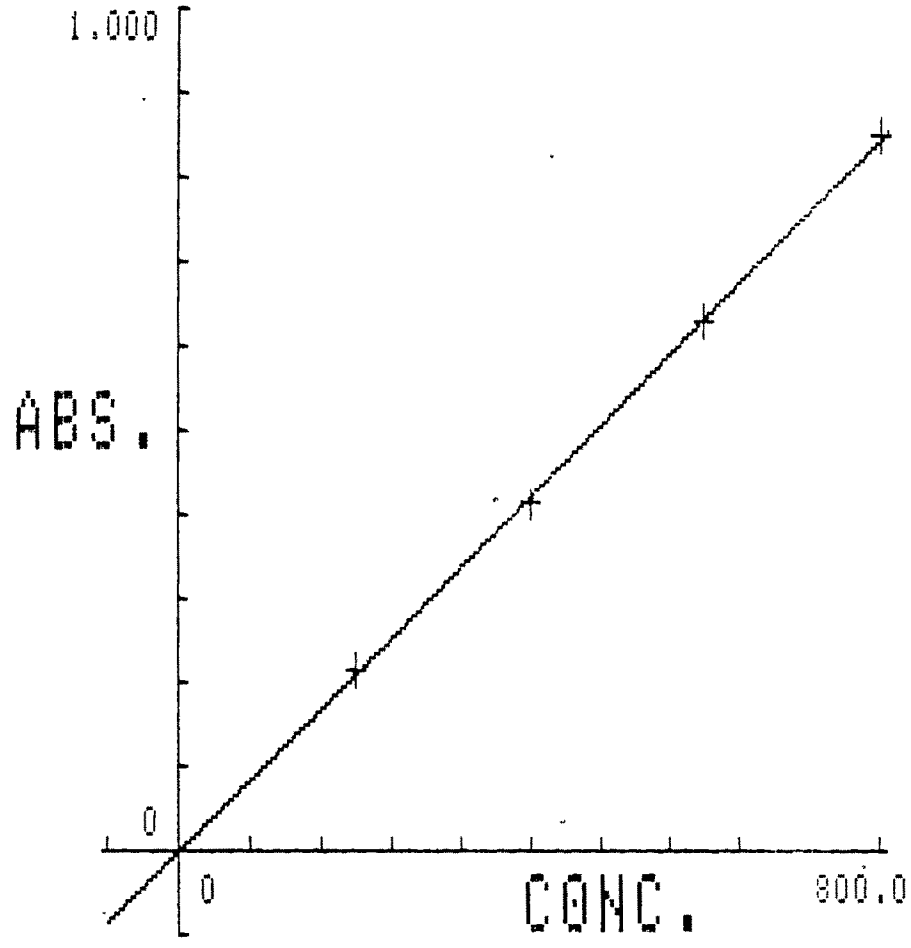


Fig. 2.14b : Linearity plot of DLZ-ferric hydroxamate method

Intereference: Common excipients found in the tablet formulation showed no interference. Moreover, desacetyl diltiazem hydrochloride and acetic acid, the products of hydrolysis of the drug, also showed no interference. Interfernece studies were carried out with both the standard reference sample of desacetyl diltiazem hydrochloride and acetic acid and also partially hydrolysed drug sample.

Reproducibility and recovery: A 5 ml volume of diltiazem hydrochloride solution (1 $mg.ml^{-1}$) was placed in each of six 25 ml flasks. The colour was developed as described under procedure for the calibration curve. The absorbance values were found to be reproducible with a RSD of 0.72%. For recovery studies, 3 ml of standard drug solution were added to 3 ml of tablet solution which had been analysed earlier. The colour of the solution was developed together with that of standard diltiazem solution. The recovery of the drug was 98.95-100.86%.

The above results show that the proposed method is simple for the analysis of DLZ and insensitive to products of its hydrolysis. The method is thus suitable for the routine analysis of diltiazem tablets and can serve as an alternative to official USP method.