CHAPTER 5 : *Fluorimetric and Titrimetric Analysis*

CHAPTER 5

FLUORIMETRIC AND TITRIMETRIC ANALYSIS

This chapter describes the details regarding fluorimetric and titrimetric determination of DLZ in formulations.

5.1 Fluorimetric method for the determination of DLZ

(Methods B-17 and B-18)

Fluorescence is the emission of light of one wavelength by a molecule, a very short time after it absorbs light of some other, usually shorter wavelength. The intensity of the emitted light is often linear over a range of low concentration.

Fluorimetric methods were developed for the determination of DLZ in tablets based on ion-pair complexation of the drug with reagents viz eosine yellow and erythrosine-y in acidic medium. The complexes were quantitativley extracted in chloroform and the fluorescence intensity was measured at 515 nm with excitation at 365 nm.

Instrumentation: Shimadzu RF-540 spectrofluorimeter and ELICO CL-53 fluorimeter were used.

Reagents: Eosine yellow and erythrosine-y were of Fluka Chemie. The reagents used were 0.3% w/v solutions of eosine yellow and erythrosine-y in water. The buffer solution of pH 5.0 was prepared by mixing 0.05 M solution of potassium hydrogen phthalate with appropriate quantity of 1 N sodium hydroxide. Chloroform and anhydrous sodium sulphate used were of AR grade.

Standard stock solution: Prepared by dissolving 50 mg of diltiazem hydrochloride in 250 ml distilled water.

5.1.1 Procedure for calibration graph:

Into a series of 125 ml separating funnels different volumes of the drug solution (200 $\mu g.ml^{-1}$, 1-5 ml) were pipetted out. The total volume was adjusted to 5 ml in all the funnels with water. To each funnel 5 ml of either eosine yellow or erythrosine-y solution were added. 25 ml of chloroform was added to all the funnels to extract the ion-pair complex. The separating funnels were shaken for 2 min and allowed to stand for the clear separation of two layers. The lower chloroform layer was passed through anhydrous sodium sulphate and the fluorescence intensity was measured at 515 nm with excitation at 365 nm.

Tablet analysis: Tablet powder equivalent to 30 mg of the drug was shaken with 250 ml water and filtered. Five ml of the filtrate was used for the method described above.

5.1.2 RESULTS AND DISCUSSION

Diltiazem hydrochloride being a basic compound reacts with acidic fluorescent dyes like eosine-y or erythrosine-y forming neutral complexes, which exhibit fluorescence, the reaction is thus beneficial for fluorimetric analysis of the drug.

DLZ has no natural fluorescence in organic solvent such as dichloromethane and chloroform. Also, solutions of these dyes in organic solvents are not fluorescent. However, upon mixing the drug and the dye solutions they produce a highly fluorescent product which reaches its maximum intensity instantaneously and remains stable for at least 2 hours.

In linearity study, calibration curves were found to be linear in the range 5 to 50 $\mu g.ml^{-1}$ of the drug concentration (Fig. 5.1). In tablet analysis, the amount of the drug corresponding to the fluorimetric reading were found out from the calibration curve and the content of DLZ in tablet was calculated using the dilution factor. The results are given in Table 5.1.

Several experiments were conducted to fix the optimum parameters like concentration of the dye, pH of the medium, ratio of aqueous to nonaqueous layer. The methods described are less expensive and rapid as compared to official HPLC method. Common excipients like starch, talc and colouring agents do not interfere in the proposed methods. However, interference was observed from desacetyl diltiazem, if present, in dosage form. Reproducibility and recovery results are satisfactory. The proposed methods can be used for the routine analysis of diltiazem tablets.

Table 5.1: Determination of DLZ using Eosine and Erythrosine.

Tablet	Labelled amount mg/tab	Amount found in mg/tab (± SD)		
		Eosine method	Erythrosine method	USP method
Lot A	30	29.32(0.86%)	29.30(0.95%)	29.27(0.65%)
Lot B	30	29.65(0.78%)	29.68(1.23%)	29.67(0.78%)
Lot C	60	59.05(1.12%)	58.92(0.86%)	58.96(0.86%)
Lot D	60	58.13(0.98%)	58.08(1.06%)	58.06(0.58%)

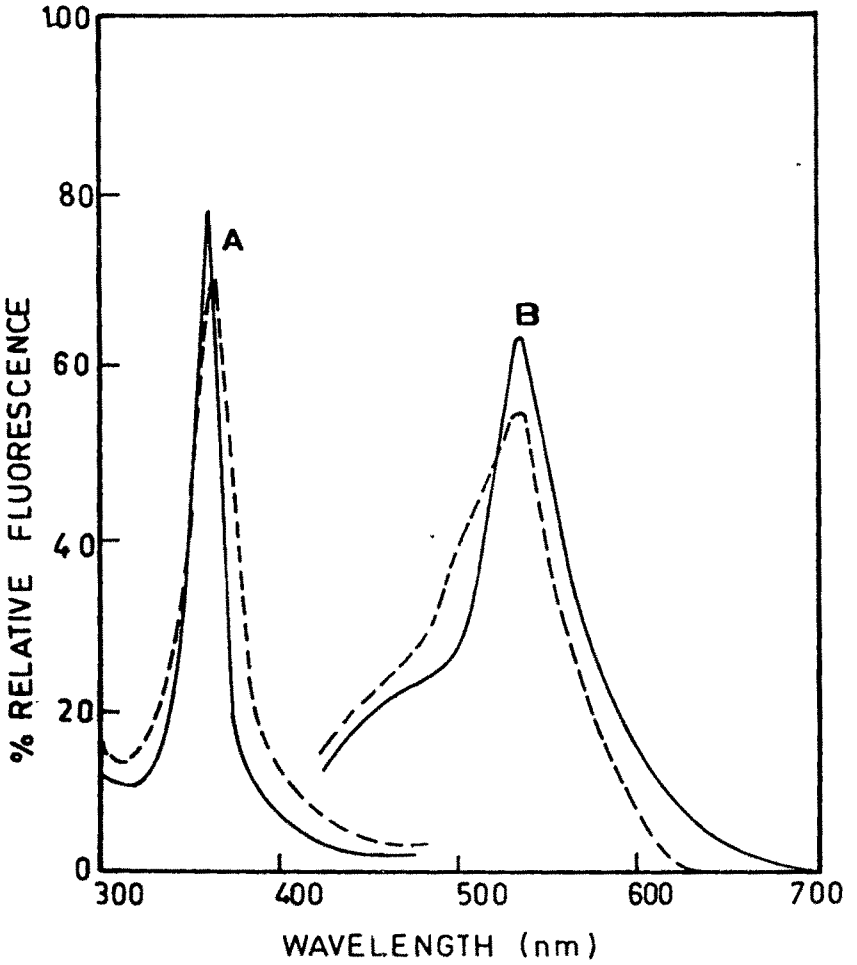

Fig. 5.1a EXCITATION SPECTRA **(A)** AND EMISSION SPECTRA **(B)** OF DLZ-EY (——) AND DLZ-ERY (----) COMPLEXES.

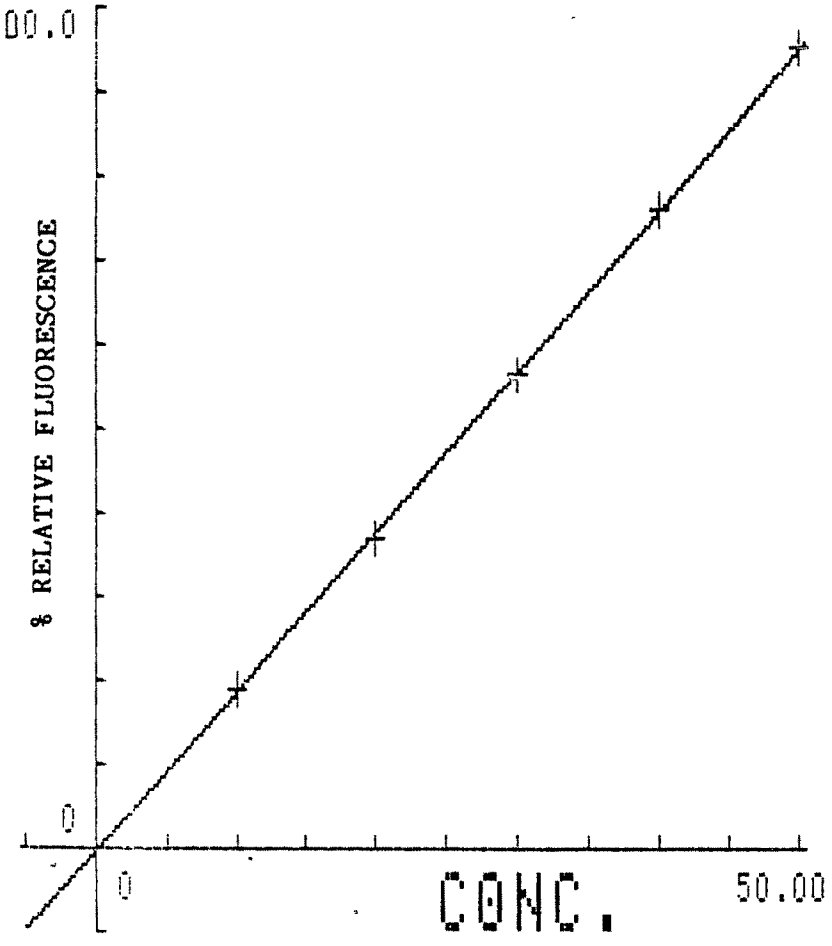

Fig. 5.1b : Linearity plot of DLZ-EY method

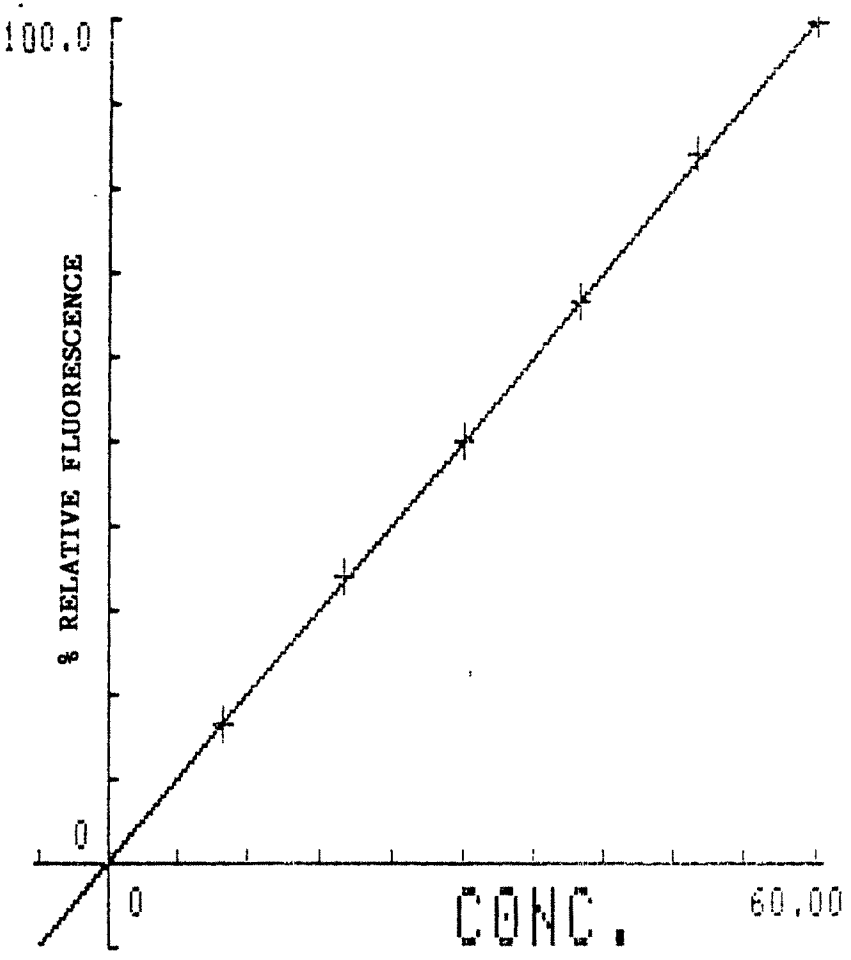

Fig. 5.1c : Linearity plot of DLZ-ERY method

5.2 Titrimetric methods for the estimation of diltiazem in tablets (Methods B-19 and B-20)

Semimicro titrimetric methods have been proposed for the determination of DLZ in tablets using anionic surface active agents viz. sodium lauryl sulphate (SLS) and dioctyl sodium sulphosuccinate (DSS) as titrants. Dimethyl yellow was used as the indicator. The titration was performed in a two phase system of water and chloroform. The salt formed during the titration was insoluble in water but soluble in chloroform. Titration was performed with vigorous shaking and the end point was indicated by the formation of golden yellow colour in chloroform layer.

Reagents: All the chemicals and reagents used were of AR grade.

SLS solution (0.005 N): 1.44 g of sodium lauryl sulphate was dissolved in 500 ml of water; 2 ml of sulphuric acid was added to get a clear solution and diluted to 100 ml with water.

DSS solution (0.005 N): 2.22 g of dioctyl sodium sulphosuccinate was dissolved in 500 ml water and diluted to 100 ml with water.

Dimethyl yellow: A solution containing 0.1 $mg.ml^{-1}$ of dimethyl yellow was prepared in alcohol.

Sulphuric acid: 2 N solution in water was used.

Standard DLZ solution: 150 mg of DLZ was dissolved in water and diluted 100 ml in a volumetric flask.

5.2.1 PROCEDURE

Standardisation: Twenty ml of standard diltiazem solution was transferred into two glass stoppered 250 ml conical flasks. Twenty ml of water was taken in two other conical flasks, one of which was to serve as the blank, the other to be used for the end point determination. Then 25 ml of chloroform, 5 ml of 2 N sulphuric acid and 1 ml of dimethyl yellow solutions were added. The solutions were titrated against SLS/DSS solutions shaking vigorously. At the end point a golden yellow colour appeared in the chloroform layer which was compared with the blank. The normality of titrant was calculated using the formula:

$$N = \frac{W}{5 \times 450.98 \times (V_S - V_B)}$$

where,

W is the weight in mg of diltiazem HCl

V_S is the average volume in ml of two titrations of standard

V_B is the volume in ml of the blank titration.

Tablet analysis:

Tablet powder equivalent to 40 mg of diltiazem HCl was transferred into glass stoppered 250 ml conical flasks. The contents were shaken with 20 ml of water and 5 ml of sulphuric acid (2 N) to get an uniform suspension. Twenty ml of chloroform and one ml of dimethyl yellow solutions were added. Titrations were carried out as described before. Content of DLZ in tablet was calculated using the formula:

$$\text{Amount of DLZ} = \frac{450.98\ (V_T - V_B)\ N\ .\ X}{W}$$

where, 450.98 = molecular weight of diltiazem HCl

N = normality of SLS or DSS solution

V_T = volume of titrant consumed by the sample

V_B = volume in ml of blank

W = weight of tablet powder in mg

X = average weight of a tablet in mg

5.2.2 RESULTS AND DISCUSSION

The proposed methods are based upon the reaction of cation moiety of drug with anionic surface active agents SLS or DSS. Earlier techniques for the determination of cationic product were based on the fact that, when cationic and anionic agents were present together in aqueous solution they will neutralise the surface activity of each other and the end point is shown by a sharp rise in surface tension. Better precision became possible when a two phase titration procedure was introduced[6]. In this method cationic product is titrated against anionic surface active agent in the presence of an indicator and a solvent, the transfer of colour from one phase to the other giving the end point.

SLS and DSS are commonly used titrants and are standardised by titration with a standard solution of compound under examination. The quality of the end point in the titration of this type may be affected by the relative solubility of quaternary ammonium compound in the two phases of the titration system. For a sharp end point, the compound being titrated should be appreciably more soluble in chloroform than in aqueous acid. The reaction of DLZ with SLS or DSS is stoichiometric and in the ratio of 1:1. The methods are rapid and reproducible.

Marketed products of DLZ were analysed using the present method and the results are given in Table 5.2.

Table 5.2: Determination of DLZ using SLS and DSS.

Formulation	Labelled amount mg/tab	% Found in (± SD)		
		SLS method	DSS method	USP method
I	30	99.30(2.0)	99.18(0.9)	99.20(1.0)
II	30	97.57(2.5)	97.60(2.5)	97.36(2.2)
III	60	98.65(1.5)	98.60(1.8)	98.34(2.3)

Normal ingredients found in tablet preparation do not interfere. However, few dye colours added in the tablets may interfere with the end point determination. These colours should be decolourised using $KMnO_4$ soluiton in acidic medium. The method is simple and sensitive and could be applied for the determination of DLZ in its formulations.