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# Spectrofluorometric estimation of aspirin and dipyridamole in pure admixtures and in dosage forms

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**Abstract**

Aspirin and dipyridamole in pure admixtures and in dosage forms have been estimated by spectrofluorometry. Aspirin (2–12 mcg $ml^{-1}$) was estimated in 1% v/v glacial acetic acid in chloroform using 246 and 345 nm for excitation and emission respectvely. Dipyridamole (2–12 mcg $ml^{-1}$) has been estimated in chloroform using 420 nm for excitation and 475 nm for emission. The non-interference of the excipients as well as the drugs in the estimation of each other, as evidenced by the results, indicate that this method may be used for the routine estimation of aspirin and dipyridamole in tablet preparations. © 1997 Elsevier Science B.V.

*Keywords:* Aspirin; Dipyridamole; Estimation; Spectrofluorometry; Tablets

## 1. Introduction

The combination of aspirin with dipyridamole is widely used as an anti-anginal preparation. Several methods have been reported for the estimation of aspirin [1–7] and some for the estimation of dipyridamole [5–9]. The present work investigates the estimation of the drugs in combined preparations without prior separation from each other and formulation excipients by spectrofluorometry.

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## 2. Experimental

### 2.1. Materials, reagents and equipment

1. Chloroform—Spectroscopic grade (Spectrochem. India)
2. Glacial acetic acid—A.R. Grade (Glaxo Laboratories, India)
3. Pure drug samples of aspirin I.P. and dipyridamole U.S.P. were obtained as gift samples from the source indicated.

The fluorescence spectra were recorded with a Jasco FP-777 scanning spectrofluorometer using 1-cm matched cuvettes.


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Table 1
Selectivity of the method for the determination of aspirin in the presence of dipyridamole by spectrofluorometry

| Composition of mixture (μg ml$^{-1}$) | | Mean value of fluorescence intensity (at 346 nm)[a] | Coefficient of variation (%) | *F* test values[b] | |
|---|---|---|---|---|---|
| ASP | DIP | | | Crit | Calc |
| 02 | 00 | 261.09 ± 6.32 | 2.42 | 3.63 | 1.76 |
| 04 | 00 | 529.60 ± 7.74 | 1.46 | 3.63 | 3.29 |
| 06 | 00 | 769.20 ± 7.46 | 0.97 | 3.63 | 2.97 |
| 08 | 00 | 1048.30 ± 8.46 | 0.80 | 3.63 | 3.14 |
| 10 | 00 | 1325.90 ± 6.18 | 0.46 | 3.63 | 2.09 |
| 12 | 00 | 1586.30 ± 6.50 | 0.41 | 3.63 | 2.26 |
| 02 | 07 | 259.20 ± 5.94 | 2.29 | 3.63 | 2.83 |
| 04 | 07 | 529.70 ± 5.36 | 1.01 | 3.63 | 3.23 |
| 06 | 07 | 773.90 ± 5.86 | 0.76 | 3.63 | 2.91 |
| 08 | 07 | 1048.40 ± 5.79 | 0.55 | 3.63 | 2.98 |
| 10 | 07 | 1329.20 ± 5.31 | 0.40 | 3.63 | 3.29 |
| 12 | 07 | 1591.50 ± 5.41 | 0.34 | 3.63 | 3.06 |

Crit, Critical value; Calc, Calculated value.
[a]Mean value of ten replicate determinations; ASP, Aspirin; DIP, Dipyridamole.
[b]Based on *F* test for non-linearity; $F_{critical} = F$ (4, 9) values from % *F* table for a tail area, $\alpha = 0.050$ (5% level of significance); $F_{calc} = S_y^2/S_s^2$ where $S_y$ is the standard error of estimate and $S_s$ is the standard deviation of a single measurement of *y*.

Table 2
Selectivity of the method for the determination of dipyridamole in the presence of dipyridamole by spectrofluorometry

| Composition of mixture (μg ml$^{-1}$) | | Mean value of fluorescence intensity (at 475 nm)[a] | Coefficient of variation (%) | *F* test values[b] | |
|---|---|---|---|---|---|
| ASP | DIP | | | Crit | Calc |
| 00 | 02 | 940.20 ± 5.71 | 0.61 | 3.63 | 3.03 |
| 00 | 04 | 1887.70 ± 5.62 | 0.30 | 3.63 | 3.14 |
| 00 | 06 | 2817.90 ± 5.96 | 0.21 | 3.63 | 2.79 |
| 00 | 08 | 3775.00 ± 4.81 | 0.13 | 3.63 | 2.84 |
| 00 | 10 | 4688.90 ± 5.66 | 0.12 | 3.63 | 3.11 |
| 00 | 12 | 5629.80 ± 5.66 | 0.10 | 3.63 | 3.11 |
| 07 | 02 | 939.80 ± 6.13 | 0.65 | 3.63 | 2.66 |
| 07 | 04 | 1888.20 ± 6.11 | 0.32 | 3.63 | 2.68 |
| 07 | 06 | 2819.00 ± 6.73 | 0.24 | 3.63 | 2.20 |
| 07 | 08 | 3774.70 ± 4.98 | 0.13 | 3.63 | 2.70 |
| 07 | 10 | 4688.00 ± 5.65 | 0.12 | 3.63 | 3.13 |
| 07 | 12 | 5630.30 ± 6.25 | 0.11 | 3.63 | 2.56 |

Crit, Critical value; Calc, Calculated value.
[a]Mean value of ten replicate determinations; ASP, Aspirin; DIP, Dipyridamole.
[b]Based on *F* test for non-linearity; $F_{critical} = F$ (4, 9) values from % *F* table for a tail area, $\alpha = 0.050$ (5% level of significance); $F_{calc} = S_y^2/S_s^2$ where $S_y$ is the standard error of estimate and $S_s$ is the standard deviation of a single measurement of *y*.

## 3. Standard and sample solutions

Four series of solutions (series A–D) of aspirin and dipyridamole were prepared by using appropriate aliquots of stock solutions of aspirin (1 mg ml$^{-1}$) in chloroform. Series A comprised solutions of aspirin of various concentrations (2–12 μg ml$^{-1}$) in 1% v/v acetic acid in chloroform and

Table 3
Regression analysis of aspirin and dipyridamole standard solutions

| Sample | Composition of sample (μg ml$^{-1}$) | | Regression[a] Equation (at 346 nm for ASP and 475 nm for DIP) | Correlation coefficient | Test for significance[b] of evidence of correlation | | Standard Error | |
|---|---|---|---|---|---|---|---|---|
| | ASP | DIP | | | Critical | Calculated | Slope | Intercept |
| Series A | 02–12 | 0 | $y = 132.71x - 8.80$ | 0.9997 | 3.747 | 98.966 | 1.341 | 10.449 |
| Series B | 0 | 02–12 | $y = 468.70x - 9.05$ | 0.9999 | 3.747 | 392.320 | 1.199 | 9.344 |
| Series C | 02–12 | 07 | $y = 133.35x - 11.47$ | 0.9998 | 3.747 | 111.751 | 1.193 | 9.294 |
| Series D | 07 | 02–12 | $y = 468.68x + 9.24$ | 0.9999 | 3.747 | 388.770 | 1.202 | 9.367 |

ASP, Aspirin; DIP, Dipyridamole.
[a]Based on six calibration values; $x$, concentration of the drug in μg ml$^{-1}$.
[b]Based on student's $t$ test at 1% significance level and 4 degrees of freedom.

series B comprised solutions of dipyridamole of various concentrations (2–12 μg ml$^{-1}$) in pure chloroform. Series C comprised of solutions of aspirin of varying concentration (2–12 μg ml$^{-1}$) along with constant concentration of dipyridamole (7 μg ml$^{-1}$) in 1% v/v acetic acid in chloroform and series D was made up of solutions containing a varying concentration of dipyridamole (2–12 μg ml$^{-1}$) and a constant concentration of aspirin (7 μg ml$^{-1}$) in pure chloroform.

Twenty tablets of each brand were finely ground and a weight of the powder equal to the average weight of the tablet was dissolved in pure chloroform and filtered (Whatman No. 1 filter paper). The first and last 5 ml of the filtrate were discarded. Appropriate volumes of aliquots of the filtrate were used to prepare sample solutions (using the solvents of 1% v/v glacial acetic acid in chloroform for estimation of aspirin and pure chloroform for the estimation of dipyridamole) containing approximately 7 μg ml$^{-1}$ of dipyridamole (and approximately 4.6 or 5.6 or 9 μg ml$^{-1}$ of aspirin). The solutions of pure drugs, their admixtures and the tablet sample solutions were scanned in a Jasco FP-777 scanning spectro fluorometer. The results of the scan have been presented in Tables 1–4 and Fig. 1 and Fig. 2.

## 4. Results and discussion

Aspirin has been reported to possess both fluorescence and phosphorescence [1]. Its hydrolytic product salicylic acid is more fluorescent than aspirin although its wavelength of excitation and emission maxima (310 and 450 nm, respectively) are different from that of aspirin. Thus far, aspirin in combined formulations has been quantified by fluorometry after its quantitative conversion to salicylic acid [3] and the estimation of aspirin by phosphorimetry requires liquid nitrogen temperatures [1]. The objective of the present investigation was to estimate aspirin as acetysalicylic acid itself (in the presence of dipyridamole and vice versa) and not as salicylic acid so that the method may be used to estimate asprin without conversion to salicylic acid [1,3]. Although aspirin fluoresces both in pure chloroform

Table 4
Assay results of aspirin and dipyridamole in commercial formulations by spectrofluorometry

| Sample | Label claim (mg/tab) | | w/w recovered[a] (%) | | Coefficient of variation (%) | |
|---|---|---|---|---|---|---|
| | ASP | DIP | ASP | DIP | ASP | DIP |
| Brand A | 60 | 75 | 99.38 ± 0.41 | 98.94 ± 0.52 | 0.41 | 0.53 |
| Brand B | 40 | 75 | 99.78 ± 0.36 | 100.14 ± 0.45 | 0.36 | 0.45 |
| Brand C | 100 | 75 | 98.90 ± 0.36 | 99.08 ± 0.47 | 0.36 | 0.47 |

ASP, Aspirin; DIP, Dipyridamole.
[a]Mean value of five determinations; assay as percentage of label claim.

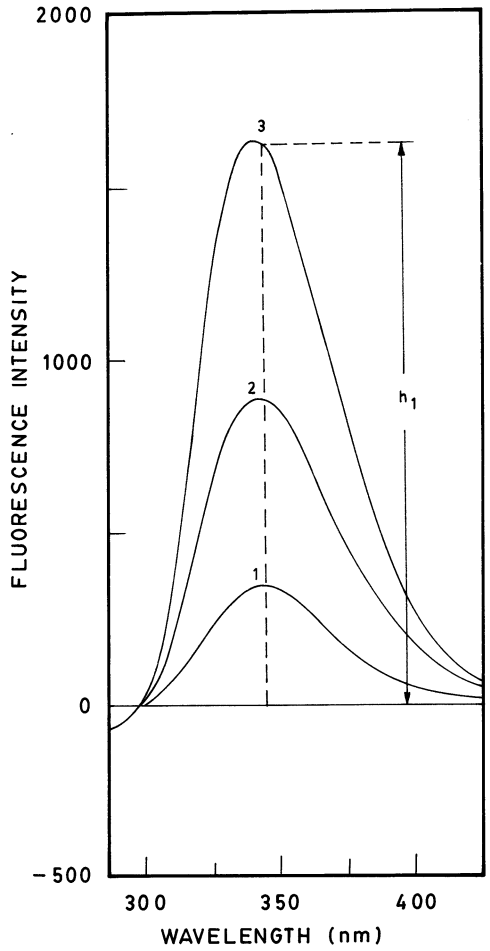


Fig. 1. Fluorescence emission spectra of aspirin; excitation at 246 nm; concentration of aspirin was 2, 6 and 12 μg ml$^{-1}$ in curves 1, 2 and 3, respectively.

as well as in 1% v/v acetic acid in chloroform, the latter has been used as a solvent for the estimation since the hydrolysis of aspirin to salicylic acid is very less in this solvent [1].

Dipyridamole also shows fluorescence in both pure as well as 1% v/v acetic acid in chloroform solvent but the rectilinear response was found only in the former solvent, probably due to the quenching effect of acetic acid on the fluorescence of dipyridamole. Hence aspirin had been quantified in 1% v/v acetic acid in chloroform. The emission spectra of pure aspirin and pure dipyridamole are given in Figs. 1 and 2 and the excitation spectra of the drugs in their respective solvents have been shown in Fig. 3.

The excitation spectra in Fig. 3 indicate that the chosen excitation wavelengths are highly specific for the particular drug. Thus, in an admixture of aspirin and dipyridamole, when aspirin was estimated by excitation at 246 nm (and emission at 345 nm), the dipyridamole does not interfere and the amplitudes of the emission spectra at 346 nm ($h_1$ in Fig. 1) were used for the determination of aspirin. Similarly, during the estimation of dipyridamole ($\lambda_{ex} = 420$ nm and $\lambda_{em} = 475$ nm) aspirin does not interfere and the amplitudes of the emission spectra of dipyridamole at 475 nm ($h_2$ in Fig. 2) have been used for the estimation of dipyridamole. The wavelength of 246 nm rather 285 nm was chosen as the excitation wavelength for aspirin since salicylic acid does not get excited

at 246 nm whereas a solution of pure salicylic acid in 1% v/v glacial acetic acid in chloroform solvent, when excited at 285 nm, emits a weak fluorescence at 346 nm (where the fluorescence of aspirin is being measured). This is clearly evidenced by the Fig. 4. The emission spectra of salicylic acid (6 μg $ml^{-1}$) with the excitation at 246 and 285 nm have been given in Fig. 4. The amplitude of the emission spectrum at 346 nm with excitation at 246 nm (curve 1 in Fig. 4) was negative whereas the emission (at 346 nm) with excitation at 285 nm (curve 2) was around 500 U. Hence, traces of salicylic acid, if present as impurity in aspirin, will not interfere with the emission of aspirin as well as dipyridamole if the wavelengths of 246 and 420 nm were used for excitation of aspirin and dipyridamole, respectively. But the fact that these readings have been recorded under a set of instrumental conditions (such as phototube response, slitwidth and scan speed) should be kept in mind. Any change in the conditions should be accompanied by a validation of the process under the new conditions since the shape, as well as fluorescence emission values, may change depending on the instrumental conditions.

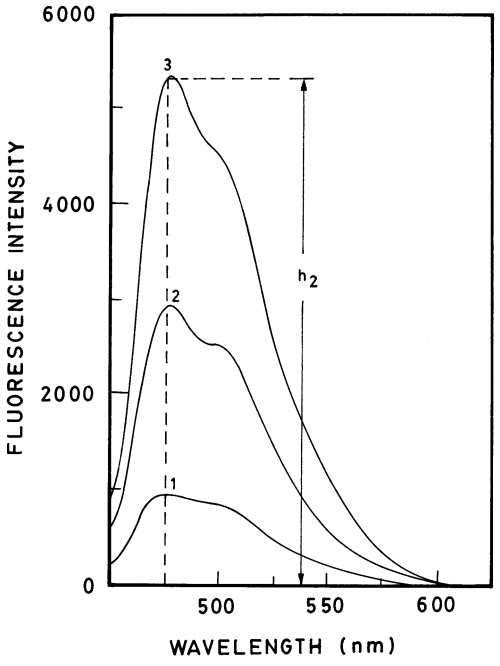


Fig. 2. Fluorescence emission spectra of dipyridamole; excitation at 420 nm; concentration of dipyridamole was 2, 6 and 12 μg $ml^{-1}$ in curves 1, 2 and 3, respectively.

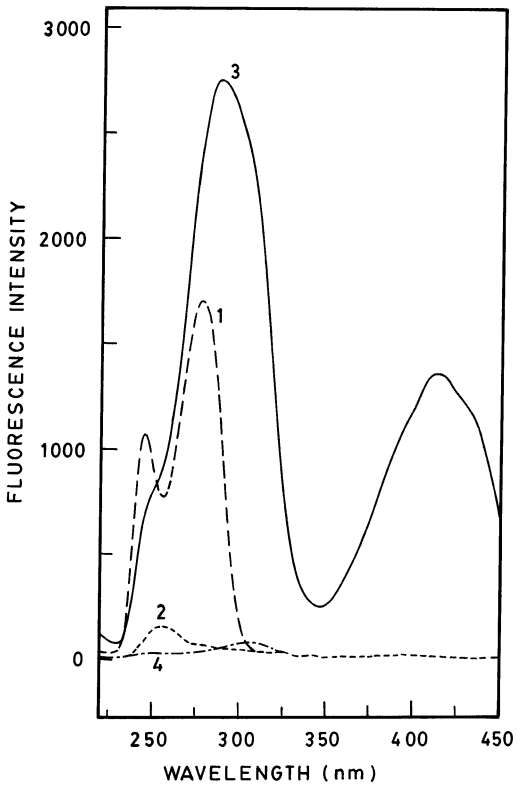


Fig. 3. Fluorescence excitation spectra of aspirin (6 μg $ml^{-1}$) in 1% v/v acetic acid in chloroform with emission at 346 nm (curve 1) and 475 nm (curve 2); excitation spectra of dipyridamole (6 μg $ml^{-1}$) in chloroform with emission at 475 nm (curve 3) and 346 nm (curve 4).

The results of statistical analysis of the spectral data have been presented in Tables 1–3. The small standard deviation values (Tables 1 and 2) indicate the high level of precision of the proposed method as well as the independence of the fluorescence emission measurement of the drugs in the presence of each other. The results of application of the *F* test for non-linearity [10] strongly indicate the existence of a linear relationship fluorescence emission values and the drug concentrations at a significance level of 5% (Tables 1 and 2) since the calculated values at significance level are less than the critical value of 3.63. The similarity of the regression equations of

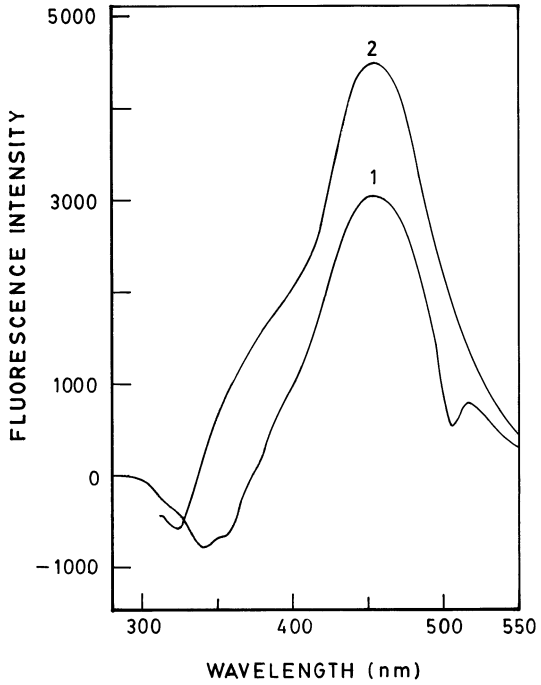


Fig. 4. Fluorescence emission spectra of pure salicylic acid (6 μg $ml^{-1}$); Curve 1 is the emission spectra with excitation at 246 nm and curve 2 is the emission spectra with excitation at 285 nm.

pure drug solutions to that of their mixtures as well as the high correlation coefficient values in the order of 0.9997–0.9999 indicate the non-interference of one drug in the fluorescence emission of the other at the chosen wavelengths (Table 3). The values of test for significance of evidence of correlation based on student's *t*-test [10] clearly confirm the existence of strong positive correlation between the concentrations of the drugs in solution and the fluorescence intensity of the respective emission spectra since the calculated values were much higher than the critical *t* values (Table 3). The negligible intercepts of the equations indicate regression through close to the origin at the chosen wavelengths (Table 3). The results of the estimation of the amounts of aspirin and dipyridamole in commercial formulations have been presented in Table 4.

The excitation and emission wavelength maxima values of aspirin and dipyridamole in the appropriate solvents were different permitting the estimation of one drug in the presence of other without interference. The data in Tables 1–3 indicate the rectilinearity, precision and reproducibility of the proposed method. Hence, in the absence of official methods for the simultaneous estimation of the drugs, the proposed method is likely to be very suitable for the routine analysis of the drugs in tablet formulations.

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