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Simultaneous Determination of Dipyridamole and Acetylsalicylic Acid in Pharmaceuticals and Biological Fluids by Synchronous and First Derivative Synchronous Fluorimetry

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Abstract:

Objective: The aim of this study was the utilization of synchronous fluorimetry using conventional and first order derivative for the simultaneous determination of dipyridamole (DPR) and acetylsalicylic acid (ASA) in pharmaceutical preparations and biological fluids.

Methods: These methods are based on the native fluorescence of both compounds at constant wavelength, the difference of 80 nm. Concentration range of 5 to 90 ng.ml^{-1} for dipyridamole and 5 to 100 ng.ml^{-1} for acetylsalicylic acid were determined by measuring the fluorescence intensity 410 nm and 310 for DPR and ASA respectively, in buffer solution of pH 7.4 and it's first derivatives.

Results: The relative standard deviations obtained by conventional and first derivatives are ±0.58 and ±0.79 for dipyridamole and ±0.76 and ±0.88 acetylsalicylic acid respectively. Correlation coefficient is about 0.9999 for both compounds. The detection limits were determined as (S/N = 3) and found to be 0.05 ng.ml^{-1} for dipyridamole and acetylsalicylic acid.

Conclusion: Our studies showed that the proposed procedures were successively applied for the simultaneous determination of both drugs in pharmaceutical preparations and in biological fluids and the results obtained are satisfactory.

Key words: synchronous fluorimetry, first derivatives, dipyridamole, acetylsalicylic acid, pharmaceuticals, biological fluids.

1. INTRODUCTION

Dipyridamole (DPR) is a platelet inhibitor that is widely used as a coronary vasodilator in patients with high blood pressure, a prophylactic agent in patients with angina pectoris and an inhibitor of platelet aggregation in various thrombogenic diseases. Aspirin (ASA) is 2- (Acetyloxy) benzoic acid, is a cyclooxygenase inhibitor which is best known as an anti-platelet drug [1] and is one of the major anti- thrombogenic agents widely used for the treatment and prevention of cerebro and cardiovascular conditions such as stroke [2]. The combination of dipyridamole and aspirin is widely used to reduce thrombosis in patients with thrombotic diseases. This antithrombotic action results from additive anti-platelet effects of both drugs. Aspirin inhibits platelet aggregation by irreversible inhibition of platelet cyclooxygenase and thus inhibiting the generation of thromboxane A2. Dipyridamole inhibits the uptake of adenosine into platelets and endothelial cells, thus decreasing the adhesion of platelets to thrombogenic surfaces [3]. Analytical methods based on high performance liquid chromatography (HPLC), HPTLC, and LC-MS [4]**.** Other methods were reported earlier for the determination of Aspirin individually and in combination with other drugs [5-9]. A few analytical procedures were also proposed for the determination of Dipyridamole in dosage forms in human plasma, serum, urine and feces [10-12]. Although the combinational use of aspirin and dipyridamole is continuously increasing, few methods were reported for the simultaneous determination of aspirin and dipyridamole using combination of liquid chromatographic and mass spectrometric detection [13]. Second order derivative spectrophometric [14], spectrofluorometric [15] and electrochemical methods [16]. The objective of this article was to develop and validate simple, robust, sensitive, reproducible and cost effective conventional synchronous fluorimetric and first order derivative methods for the estimation of dipyridamole and aspirin in pharmaceutical dosage forms and biological fluids.

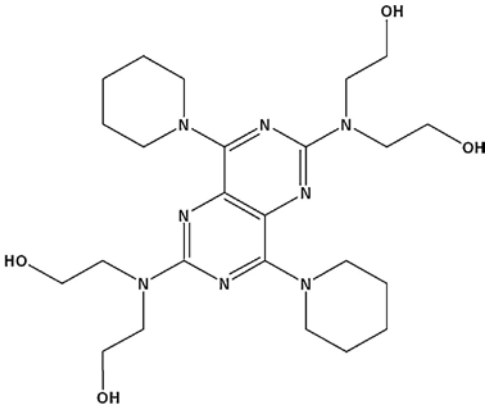

Figure 1: Structural formula of dipyridamole

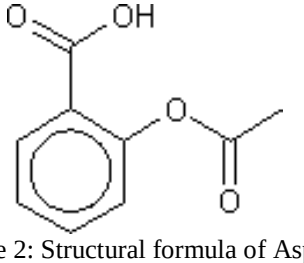

Figure 2: Structural formula of Aspirin

MATERIAL AND METHODS:

Apparatus

The fluorescence spectra and measurements were recorded using a Perkin-Elmer UK model LS 45 luminescence spectrometer, equipped with a 150 Watt Xenon arc lamp, grating excitation and emission monochromators for all measurements and a Perkin-Elmer recorder. Slit widths for both monochromators were set at $\Delta\lambda$ 10 nm. A 1 cm quartz cell was used. Derivative spectra can be evaluated using Fluorescence Data Manger (FLDM) software.

A pH meter (Model pHS-3C, Shanghai Leici instruments Factory, China) was used for pH adjustment.

Materials and reagents:

All the reagents used were of Analytical or pharmaceutical grade. Dipyridamole and acetylsalicylic acid were supplied by Böehringer Ingelheim Co. (Germany). Pharmaceutical dosage form (tablets) were obtained from commercial sources. Blood samples were kindly supplied by the blood bank of Mansoura University Hospital. Dipyridamole and acetylsalicylic acid stock solutions are prepared in a concentration of 2μgml^{-1} methanolic solution by serial dilution of 20 mg of each drug in 100 ml methanol.

Phosphate buffer solutions (0.2 M) in the pH range of 3 – 8 were prepared in distilled water, the pH of these buffer solutions were finally adjusted using di-sodium hydrogen phosphate or concentrated phosphoric acid solutions.

Procedures:

Calibration curve:

Aliquot volumes of stock solutions containing 50-900 ng dipyridamole or 50 to1000 ng acetylsalicylic acid were transferred into 10 ml volumetric flasks, followed by addition of 2 ml of 0.2 M phosphate buffer pH 7.4. The solutions were mixed well and the volume was completed to the mark with methanol. The fluorescence intensity was recorded at 310 and 410 nm versus blank. A blank experiment was carried out under the same conditions without drug. The fluorescence intensity or first derivatives were plotted against the drug concentrations. Calibration curves were plotted and the corresponding regression equations were derived.

Determination of DPR and ASA in tablets:

10 capsules were weighed and pulverized, and a quantity of the powder equivalent to 20 mg of dipyridamole or acetylsalicylic acid was transferred into a small conical flask. Extracted quantitatively with 4 x 20 ml of ethyl alcohol, filtered into a 100 ml volumetric flask, the volume was completed to the mark with the same solvent. 1 ml containing 10 □g of each compounds (after serial dilution) was transferred into a 10 ml volumetric flask. The procedure was completed as described under calibration curve. The concentration of each drugs was determined from the corresponding derived regression equation.

Determination of dipyridamole and acetylsalicylic acid in spiked serum:

To a 5 ml of human serum spiked with dipyridamole and acetylsalicylic acid as shown in table (2), 2ml of 10 % w/V trichloroacetic acid solution (TCA) was added, homogenized for 1 min, and then centrifuged for 2 min at 3500 rpm. 1 ml of the supernatant was transferred into a 10 ml flask. The procedure as described under calibration curve was followed up. Then the concentration of each drug was determined using the corresponding derived regression equations

RESULTS

Different experimental parameters affecting the formation and stability of the fluorescence of the studied compounds were carefully studied and optimized. Such factors were changed individually while others were kept constant. These factors included $\Delta\lambda$ selection, pH, type and volume of the diluting solvent, stability time and ionic strength of sodium chloride and the following results were recorded:

Selection of optimum $\Delta\lambda$

The synchronous fluorescence spectra of DPR with ASA were recorded using different $\Delta\lambda$. The optimum $\Delta\lambda$ value is very important for performing synchronous fluorescence scanning technique concerning resolution, sensitivity and features. It can directly influence spectral shape, band width and signal value. For this reason a wide range of $\Delta\lambda$ (20, 40, 60, 80, 100 and 120 nm) was examined. When $\Delta\lambda$ is less than 80 nm, the spectra shape is irregular and the fluorescence intensity is very weak. When $\Delta\lambda$ is more than 80 nm, poor separation of the two peaks was obtained. Therefore, $\Delta\lambda$ of 80 nm was chosen as the optimal one for the separation of the mixture of DPR with ASA, since it resulted in two distinct peaks with good shape and to minimize the spectral interference caused by each compound in the mixture Figures(3-9).

Effect of pH.

The effect of pH on the fluorescence intensity of DPR and ASA was studied using different buffer solutions covering the whole pH range, such as acetate buffer, borate and phosphate buffers over the pH range of 3 -11was studied. The results showed that the relative fluorescence intensities of the studied compounds change with pH. It is seen that the maximum relative fluorescence intensity of DPR and ASA appear at pH 7.4 using phosphate buffer. Other buffers with same pH produce less relative fluorescence intensity. The same results have been reported [17].

Effect of diluting solvent

Dilution with different solvents such as water, methanol, ethanol, isopropanol, dimethyl sulfoxide (DMSO) and dimethyl formamide (DMF) as attempted. Methanol proved to be the solvent of choice with higher fluorescence intensity and results reproducibility

The effect of volume of methanol was also studied. The results showed that relative fluorescence intensity of DPR and ASA have no great change over the range of 4–7 mL of methanol. So, 5 mL of methanol was selected as the optimum volume of diluting solvent for determination of DRP with ASA.

Effect of time

The effect of time on the development and stability of the fluorescence intensity of the drugs was also studied. It was found that the fluorescence intensity developed rapidly and remained stable for more than 2 hours, so, the method is highly rapid relative to reported methods [13-16].

Effect of ionic strength

The impact of ionic strength on the system was also studied by using different concentrations of sodium chloride (NaCl) ranging from 5 x 10^{-3} M to 0.1 M. NaCl has been used as a counter ion to study ionic strength since sodium ions have higher adsorption over the negative charge on the molecule and chloride ions have the same effect. The results showed that there was no effect of NaCl concentration on the fluorescence intensity of the studied drugs.

Table I: Proposed method for the determination of dipyridamole and acetylsalicylic acid in pure forms by synchronous and first derivative synchronous fluorimetry

Drug	Method	Slope	Intercept	R	Mean %* ± RSD
Dipyridamol	Synchronous	10.3789	0.7897	0.9999	99.77±0.58
Dipyridamol	First derivatives	2.6792	0.1352	0.9999	100.63±0.76
Acetylsalicylic acid	Synchronous	8.4560	0.5717	0.9999	99.74±0.79
Acetylsalicylic acid	First derivatives	1.4560	-0.2812	0.9999	99.79±0.88

Average of seven triplicate determinations covering concentration range

Where, r is the correlation coefficient and RSD is the relative standard deviation

Table II: Application of the proposed method to the determination of dipyridamole by synchronous and first derivative synchronous fluorimetry

Preparation	Proposed method			Reference method [19]
	□g taken	□g found	% Recovery	% Recovery
Dipyridamole (Synchronous)	5.0 10.0 20.0 45.0 60.0 80.0 90.0 X± SD	4.934 9.944 19.966 45.207 60.144 79.896 89.910	98.68 99.44 99.83 100.46 100.24 99.87 99.90 99.77±0.58	99.89±1.34
Dipyridamole (First derivatives)	5.0 10.0 20.0 45.0 60.0 80.0 90.0 X± SD	5.089 10.128 20.206 44.843 60.144 80.296 90.027	101.78 101..28 101.03 98.95 100.00 100.42 100.03 100.63±0.76	99.89±1.34

Average of seven triplicate determinations covering concentration range.

Table III: Application of the proposed method to the determination of acetylsalicylic acid by synchronous and first derivative synchronous fluorimetry

Preparation	Proposed method			Reference method [19]
	□g taken	□g found	% Recovery	% Recovery
Acetylsalicylic acid (Synchronous)	5.0 10.0 25.0 40.0 60.0 80.0 100.0 X± SD	4.900 9.984 25.003 40.14 60.008 80.112 99.860	98..00 99.84 100.01 100.35 100.01 100.14 99.86 99.74±0.79	99.89±1.34
Acetylsalicylic acid (First derivatives)	5 10 25 40 60 80 100 X± SD	5.001 9.809 24.918 40.028 60.630 79.864 99.780	100.02 98.09 99.67 100.07 101.05 99.63 99.78 99.79±.0.88	99.89±1.34

Average of seven triplicate determinations covering concentration range

Table IV: Application of the proposed Method to the determination of DPR and ASA in *Persantin®, 25 mg* tablets and spiked blood serum by proposed method and reference mthod[19]

Dosage form	Proposed method, % recovery ± RSD Synchronous fluorimetry	Proposed method, % recovery ± RSD, first derivative Synchronous fluorimetry	Reference method[19] % recovery ± RSD
Persantin®, 25 mg tablets DPR	101.35±1.34	102.67 ±1.65	100.96±1.89
Persantin®, 25 mg tablets ASA	99.96 ± 1.58	100.31 ±1.79	100.76±1.98
Spiked blood serum DPR	103.98± 2.34	102± 2.67	103.74±2.87
Spiked blood serum ASA	104.78 ± 2.74	103.96 ±.1.65	104.95± 2.31

Average of five triplicate determinations

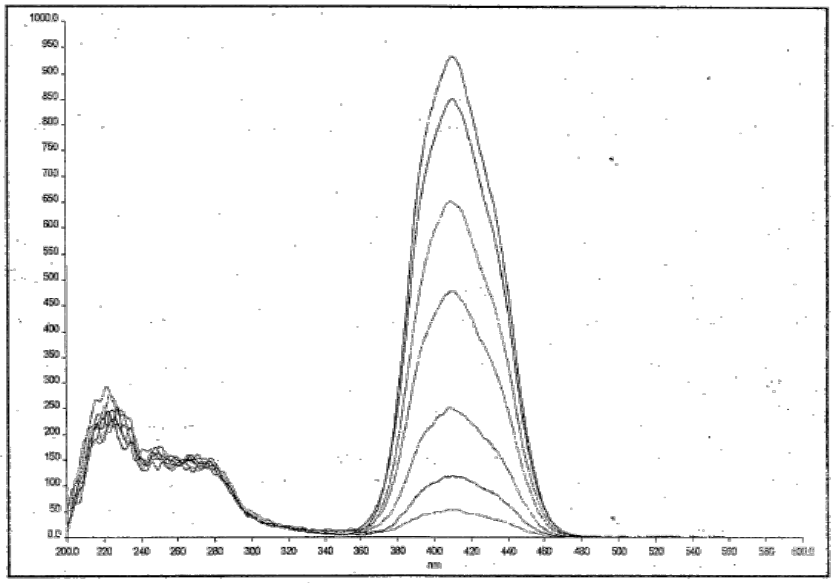

Figure 3: Conventional Synchronous Fluorescence 5.0, 10.0,20.0, 45.0 , 60.0, 80.0 and 90.0 ng/mL of DPR.

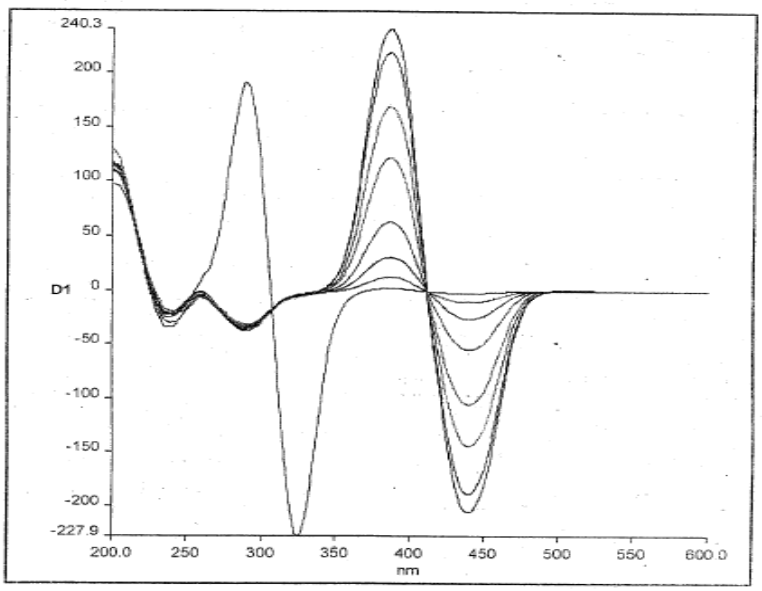

Figure 4: First derivatives Synchronous Fluorescence 5.0, 10.0, 20.0, 45.0, 60.0, 80 .0and 90.0 ng/mL of DPR.

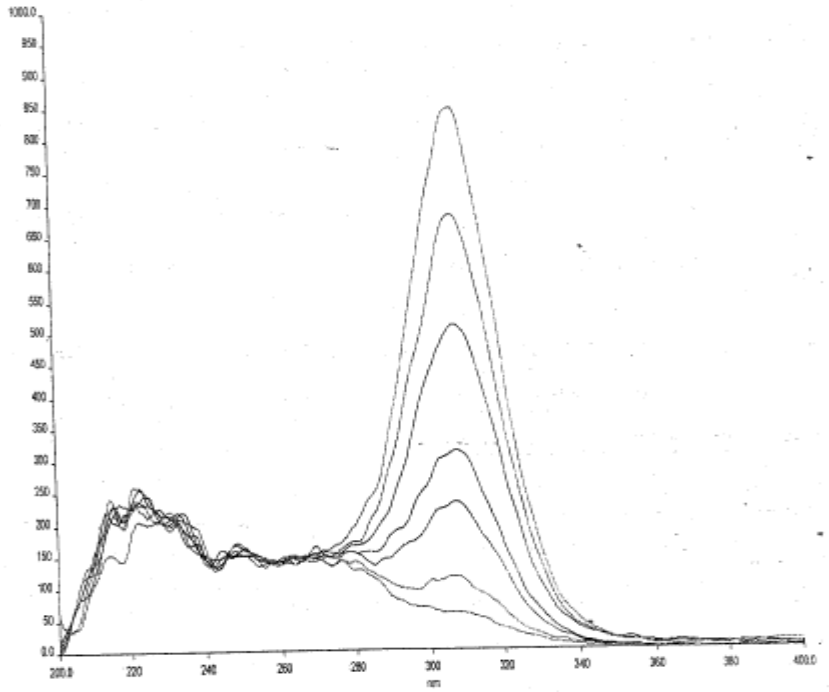

Figure 5 :Conventional Synchronous Fluorescence 5.0, 10.0, 25.0, 45.0 , 60.0, 80.0 and 100.0 ng/mL of ASA.

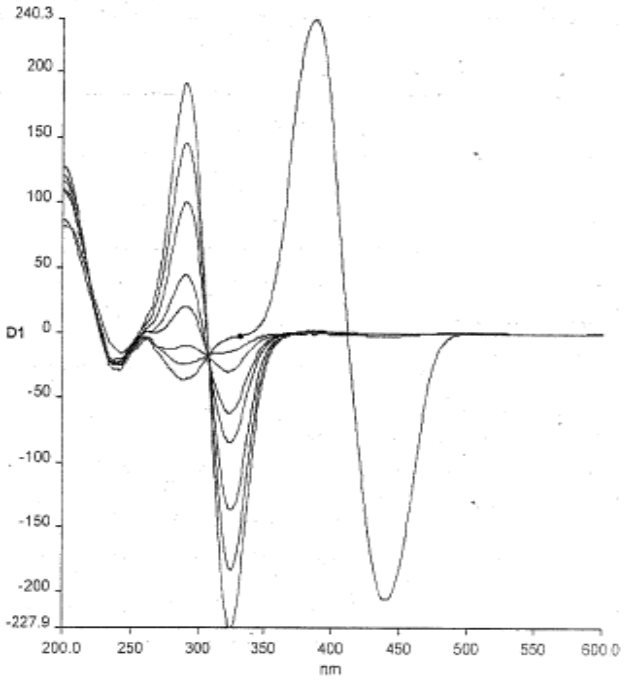

Figure 6: First derivatives Synchronous Fluorescence 5.0, 10.0, 25.0,45.0 60.0, 80.0 and 100.0 ng/mL of ASA.

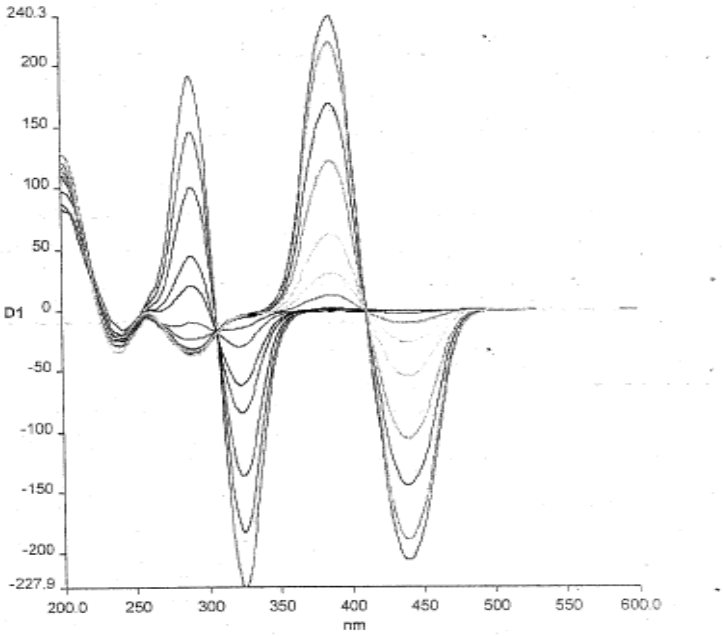

Figure 7 : First derivatives Synchronous Fluorescence of 5.0, 10.0, 20.0, 45.0, 60.0, 80.0 and 90.0 ng/mL of DPR and 5.0, 10.0, 25.0,45.0, 60.0, 80.0 and 100.0 ng/mL of ASA.

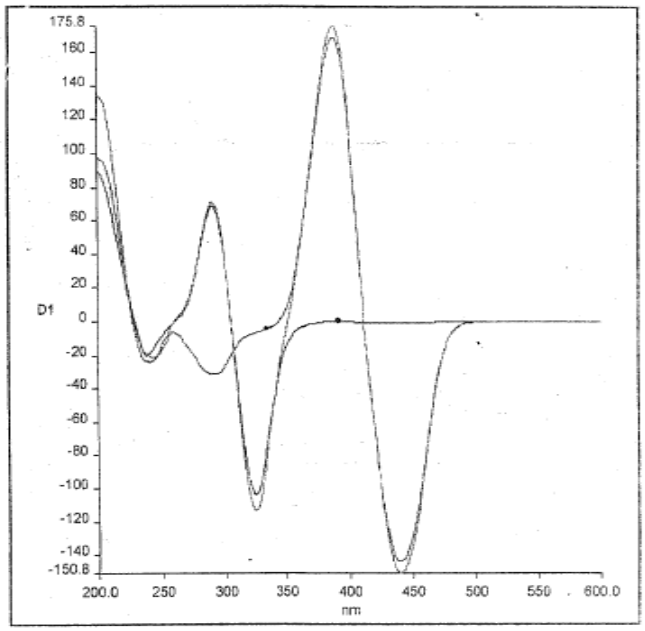

Figure 8 : Zero crossing synchronous Fluorescence of the binary mixture of 60.0 ng/mL DPR and 40.0 ng/mL of ASA .

DISCUSSION

The use of synchronous fluorimetry and its first derivatives for the determination of pharmaceutical substances has been widely applied due to its simplicity and avoidance of problems concerned with the preliminary treatments of samples. Moreover, these methods are rapid, selective and highly sensitive [17,18].

Figure 3 shows the synchronous fluorescence spectra of DPR, the maximum relativities of fluorescence intensity appears at 410 nm. Figure 4 shows the first derivatives of synchronous fluorimetry of a serial concentration of DPR from 5-90 ng//ml, the statistical analysis of the results is shown in table 1. It can be concluded from the results that synchronous fluorimetry and first derivatives are accurate,

precise and reproducible for the determination of DPR, since the correlation coefficient is 0.9999 and mean recoveries are 99.79 and 100.63 with relative standard deviation ±0.58.and ± 0.76 respectively.

While Figure 5 and 6 show the synchronous fluorescence spectra of ASA and its first derivatives, respectively, of a serial concentration of ASA with concentration range 5-100 ng//ml, the maximum relativities fluorescence intensity appears at 310 nm. The statistical analysis of the results is shown in table I, concluding that, these methods accurate, precise and reproducible for the determination of ASA since the correlation coefficient is 0.9999 and mean recoveries are 99.74 and 99.97 with relative standard deviation ±0.79.and ±0.88 respectively.

Figure 7 demonstrates the first derivatives synchronous fluorimetry for DPR and aspirin at the maximum fluorescence intensities appears at the same wavelength without change. In one preferred embodiment of a zero crossing detecting circuit in accordance with the invention of a read head is used to sense the data signal in the form of a magnetic recording. The output of the read head is differentiated to reproduce the data signal as recorded on the magnetic medium [20]. Moreover, Figure 8 shows zero crossing synchronous fluorimetry of binary mixture 60 ng / ml DPR and 40 ng/ml ASA, respectively, the produced data signal for both figures is compared with a reference signal representing the zero level of the data signal so as to produce a bi-level signal which changes level upon the occurrence of each zero crossing, so no interferences between the two signals, hence so, the method proved to be highly selective. Moreover, the method is highly sensitive, since, the detection limits were determined as (S/N = 3) were found to be 0.05 $ngml^{-1}$ for both dipyridamole and acetylsalicylic acid.

Table II and III show the assay results of DPR and ASA, respectively, by proposed synchronous fluorimetry and its first derivative and the reported native direct fluorimetric method [19], the proposed method proved to be in a good agreement with native fluorimetric method, moreover the proposed methods are more sensitive and selective than the reported method.

Table III shows the assay results of the determination of DPR and ASA in Persantin®, 25 mg tablets and blood serum by proposed method and reference method [19]. Tablet ingredients other than DPR and ASA, such as stabilizers and additives did not interfere with the recommended methods. Serum contents other than DPR and ASA did not interfere with the recommended procedures. The proposed method can be used for quality control of the binary mixture of DPR and ASA

CONCLUSION:

In summary, from the forgoing discussion synchronous fluorimetry and first derivative technique were applied successfully for the determination of dipyridamole and Aspirin in bulk, pharmaceutical preparations and blood serum and the results obtained were satisfactory.

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