Original Research Article

A profoundly touchy approved spectrofluorimetric approach for assurance of ixabepilone as anticancer drug by utilizing its quenching impact on acetoxymercuric fluorescein reagent

Abstract

A dependable, sensitive, basic and cheap spectrofluorimetric approach has been created for test of sulfur-containing drug; ixabepilone in bulk powder, vials and human plasma. The approach depends on the quenching effect of ixabepilone on the fluorescence intensity of acetoxymercuric fluorescene (AMF) reagent at λem of 530 nm and λex of 500 nm. Parameters which will control the reaction such as pH, AMF solution concentration, temperature, time and solvents were examined and optimized. According to the optimized conditions, the proposed approach was practiced over the concentration area of 20-100 ng mL-1 with adequate linearity (r = 0.9998). The developed approach was approved confirming to ICH rules in terms of accuracy, precision, linearity, LOD and LOQ. The proposed approach was practiced to analyze ixabepilone in Ixempra® vials with satisfactory recovery % of 99.89 and RSE% of 1.24. The results achieved were compared to those achieved by an already reported HPLC approach.

Keywords: Spectrofluorimetric; Ixabepilone as anticancer; Acetoxymercuric fluorescein reagent; Vials; Human serum

1. Introduction

Ixabepilone (IXA) is (1S,3S,7S,10R,11S,12S,16R)-7,11-dihydroxy 8, 8, 10, 12, 16-pentamethyl-3-[(E)-1-(2-methyl-1,3-thiazol-4-yl)prop-1-en-2-yl]-17-oxa-4-azabicyclo [14.1.0] heptadecane-5,9-dione [1] (Figure 1) is an orally bioavailable semisynthetic analogue of epothilone B with antineoplastic activity, a natural chemical compound produced by Sorangium cellulosum [2]. Epothilone B itself might not be created as a pharmaceutical drug since of low metabolic stability and pharmacokinetics [3]. The epothilones parallel taxanes in that they connect to β-tubulin and trigger microtubule nucleation at numerous spots farther from the centriole. This chaotic microtubule stabilization triggers cell-cycle capture at the G2-M interface and apoptosis. Epothilones connect to a location definite from that of taxanes. In colon cancer cell lines, p53 and Bax trigger apoptosis in ixabepilone-treated cells. In vitro application, advise that ixabepilone is less inclined to P-glycoprotein-mediated multidrug resistance when compared to taxanes. Other instrument involved in epothilone resistance incorporate mutation of the β-tubulin active site of binding and upregulation of isoforms of β-tubulin [4]. Ixabepilone was constructed through medicinal chemistry advanced upon these properconnects [3]. It is very potent, able of harming cancer cells in exceptionally low concentrations, and holds action in cases where tumor cells are heartless to taxane brand drugs [5]. As with the taxanes and other agents that target tubulin, the epothilones, counting ixabepilone, connect to the b-tubulin subunits of microtubules to initiate microtubule polymerization and stabilization, which lead to capture of cells within the G2-M stage of the cell cycle and the initiation of apoptosis (Figure 2). A lack of chemical methods deduced for determining of IXA, rather than LC [6-8], appeared in the literature as enlisted in this review.

Non-fluorescent compounds holding sulphide or sulphydryl moieties, were determined quantitatively with acetoxymercuric fluorescein (AMF), a mercuric acetate substituted fluorescein; which consider a widely used fluorescent agent, depending on the reaction of Hg^{2+} incorporated in (AMF) with the sulfur containing groups in the analyzed compounds (Fig. 3) [9], this reaction decreases the intensity of the (AMF)fluorescence that measured quantitatively with the tested compounds [10-12]. Many compounds successfully determined quantitively using this method such as mesna, acetylcysteine, timonacic corrosive [13], penicillamine [14] and mirabegron [15]. In this study, the reaction of IXB with its sulfide group with AMF and the quenching effect on the fluorescence were measured spectrofluorimetricaly at (ƛ em 530 nm) [9]. It is worth to mention that there is no publication conducted for the IXA assay spectrofluorimetricaly either in bulk, dosages forms or human biological fluids.

This work aimed to construct a spectrofluorimetric method privileged with validity, sensitivity, simplicity and reliability along with the advantages of being costly effective and rapid when compared with other widely used techniques, for the purpose of quantitative determination of IXA in bulk, pharmaceutical dosages forms or human biological fluids.

In spite of the non-existence of a procedure conducted for the assay of IXA spectrofluorimetricaly until now, more improvements needed eagerly to attain more suitable conditions and better analytical performance.

2. Experimental

2.1. Instrumentation

All spectrofluorimetric measurements were carried out on Agilent Cary Overshadow Fluorescence Spectrofluorimeter (USA); prepared with a 150 W xenon streak light and 1 cm quartz cell were utilized. The excitation and emanation opening width was 10 nm, worked with Cary overshadow check application program adaptation 1.2. pH estimations were made with HANNA pH 211 Chip pH Meter with two fold intersection glass anode. Digital pH meter 3310 Jenway.

2.2. Materials and reagents

Ixabepilone (IXA) was gently donated from Bristol-Myers Squibb (USA, Akhenaton office (Egypt)). Acetoxymercuric fluorescein (AMF), 1x10-4 M solution was arranged by dissolving 82.3 mg of AMF powder in 20 mL of 0.1 N NaOH, weakened with 100 mL of 0.1 M boric acid solution and the volume was completed to 1.0 L utilizing refined water [9]. It is suggested that the solution be kept secured from light in fridge. Britton Robinson buffer utilized in optimization trials was arranged by infusing match volumes of boric acid (0.1 M), acetic acid (0.1 M) and phosphoric acid (0.1 M) in a 100 mL volumetric flask at that point the pH was adapted within the wanted area (5-9) by including acceptable volumes of sodium hydroxide (0.1 N) [16]. Methanol, ethanol, isopropanol, chloroform and dimethylformamide (DMF) solvents were acquired from El-Nasr Co. Egypt. All reagents and solvents utilized were of analytical class. A fresh arranged bi-distilled water was utilized through all tests. Ixempra® vials 45 mg per vials (Batch no. 69019) is a brand of Bristol-Myers Squibb (USA, Akhenaton office (Egypt). Plasma sampling were achieved from Minia University Hospital, blood bank, Minia, Egypt and were kept solidified until utilize after delicate defrosting.

2.3. Preparation of standard stock solution

Standard stock solution of the drug (0.25 mg mL^{-1}) was prepared by dissolving 0.025 of IXA in 100 mL methanol and kept in refrigerator protected from light.

2.4. Spectrofluorimetric procedure and construction of the calibrated curve

The proposed approach was practiced beneath the optimized conditions that will be examined afterward. Precisely measured volumes of the stock standard solution were relocated into a set of 10-mL volumetric flasks to achieve a IXA concentration area of 20-100 ng mL-1 followed by the inclusion of 1.0 mL of 1 AMF reagent. The solutions were blended well applying a vortex and left to stand at room temperature for 10 min. Each flask was weakened quantitatively with methanol. The fluorescence intensity was detected at λem of 530 nm after excitation at λex 500 nm. At that point the fluorescence change was determined by subtracting the fluorescence intensity of the reaction admixtures from the comparing values of so also treated blank (a solution contains 1.0 mL of AMF reagent and weakened with methanol). A calibration curve detailing the fluorescence contrasts at λem 530 nm to the comparing drug concentrations in ng mL-1 was developed.

2.5. Application procedures

2.5.1. Procedure for pharmaceutical preparation

Ixempra ® vials test: (45 mg per vial). An aliquot of 1 mL from the blended substance of Ixempra ® vials was precisely relocated to a 100 mL volumetric flask and broken down in methanol, at that point the volume was completed to the line with methanol. 0.5 mL of this solution was weakened with methanol to earn an eventual IXA working solution concentration. The method was at that point completed as already defined.

2.5.2. Procedures for spiked human plasma

One-milliliter aliquots of plasma tests were delocated into two solution of centrifuge tubes. The plasma tests were spiked with 0.1, 0.2 and 0.3 mL from 12.5 mg% stock solution of IXA. The tubes were blended well by employing a vortex blender. The solutions were deproteinized twice each with 3 mL acetonitrile taken after by centrifugation for 15 min at 8000 rpm. The centrifugates were delocated to clean centrifuge tubes and vaporized. The residues were transformed in to methanol and delocated to 5 mL volumetric flasks and the volumes were adapted to the line with the same solvent. Aliquots of 2 mL from each solution were delocated to a 25 mL volumetric flask, the desired volumes of buffer and AMF reagent were included and the volume was completed to the line with methanol. A step weakening was achieved by delocating 1 mL from the flask of the reaction blend to a 100 mL volumetric flask and weakening to volume was made by methanol. The relative fluorescence intensities were measured utilizing the previous cited fluorescence method and subtracted from the comparing resultes of a essentially treated blank.

3. Results and discussion

Acetoxymercuric fluorescein (AMF) is a mercuriated derivative of fluorescein (a reagent with green fluorescence) [17], which combine with mercury complexing agents such as sulfides, arising in quenching of its fluorescence. This reaction is called the Wronski reaction [18]. Upon the reaction of compounds having sulfur with AMF, the last mentioned is changed over to weak fluorescent ones. This may be because of available alter within the chromophoric structure of the reagent particle. For encourage clarification of the reaction mechanisms, it was presumed that anions which can shape stable Hg2+ complexes would replace the acetoxy moiety in AMF structure to make a solid chelate with Hg2+ cation [17]. The proposed reaction pathway is shown in scheme 1. Figure 4 appears the fluorescence

quenching of the reagent within the nearness of IXA. The quenching pathway was examined by developing Stern-Volmer plot. It is a plot that appears a connection between (Io/I) and the quencher concentration. A linear curve was achieved upon plotting (Io/I) against concentration of the drug which demonstrates either inactive or energetic quenching happens in an inactive mechanism, as the quencher got to be a portion of the complex shaped amid the chemical reaction agreeing to (Eq. (1)) which speaks to a ground-state quenching model [19, 20]. This association constant Ka was determined and it is 0.1079. Io/I = 1 + Ka[Q] (1) Io is the fluorescence intensity of AMF in nonattendance of quencher whereas I is its fluorescence intensity in nearness of the quencher. Ka is the association constant and [Q] is concentration of the quencher (drug) [19].

3.1. Reaction stoichiometry

The Continous Variation Method (Job's Approach) [20] has been generally utilized with isomolar solutions to examine the complexation cases in these solutions and to decide the transcendent complexes of the reaction. It was accepted in this work to examine the reaction stoichiometry between IXA and AMF. Iso-molar concentrations of IXA and AMF (1 x 10−4 M) solutions were arranged. Precisely measured various volumes from (1 x 10−4 M) stocks of each IXA and AMF were included together into a set of test tubes in numerous proportions to get a volume of 5 mL. A connection between the achieved fluorescence difference and the proportion between the drug and the reagent was outlined in Job's plot (Fig. 5). It showed that 2.0 mol of IXA were required to full the quenching reaction of 1.0 mol of AMF, in this way the (drug: AMF) stoichiometric proportion in a total reaction was (2:1). This reaction is a complexation reaction between Hg2+ in AMF

and sulfur moiety in IXA. The achieved stoichiometric proportion can be clarified by the trade of two acetoxy moities in AMF by two moles of IXA [17] Scheme 1.

3.2. Optimization of the reaction conditions

Various parameters influencing the reaction were optimized to have the most senstivity, counting concentration of AMF reagent solution, temperature, ideal pH, time and weakening solvents. The resultes of optimization of the reaction parameters are appeared in Tables 1 & 2.

3.2.1. Effect of AMF reagent solution concentration

The impact of AMF solution concentration was considered utilizing various volumes (0.1–2 mL) of 1 x 10−4 M AMF solution to respond with a certain concentration of the drug in a solution of 10-mL volumetric flasks. The flasks' substance were blended and completed to the line with methanol and cleared out to stand for 10 min at room temperature. The fluorescence contrast was observed, at λem 530 nm utilizing λex 500 nm, for each test solution against a fresh prepared blank solution for each estimation. The connection between AMF volume and the fluorescence contrast of the reaction blend was shown to in (Fig. 6). It uncovered that; 1.0 ± 0.2 mL of 1 x10−4 M AMF was appropriate for reaction.

3.2.2. Effect of temperature

The ideal temperature for total quenching was considered by warming the reaction blend at various temperatures (40–100 °C) whereas keeping all other parameters consistent. The impact of the utilized temperature on the fluorescence quenching is shown in (Fig. 7). This appeared that, the greatest fluorescence quenching was achieved at room temperature, whereas it remained nearly consistent when the temperature was raised up to 60 °C. At

temperatures over 60 °C and up to 100 °C, the fluorescence contrast diminished. The diminish in fluorescence quenching at great temperature may be due to the separation of the shaped weak complexes that are greatly important for quenching the fluorescence [21].

3.2.3. Effect of pH

The pH plays a vital part within the sensitivity of this reaction. The impact of pH on quenching the fluorescence was examined in the pH area (5–9) utilizing the universal Britton Robinson buffer. The initial pH of the reaction blend was measured and it was 6.4. The connection between various pH and comparing fluorescence contrast in (Fig. 8) appeared that the most extreme senstivity was achieved within the solution's pH 6.4. This data is due to the reality that at pH ranges from 6 to 7, AMF appeared exceptionally solid fluorescence. This could be due to the nearness of AMF as a doubly charged anion. In this way the greatest fluorescence intensity of AMF reagent is achieved at pH 6.40. It was moreover found that upon diminishing the pH underneath 6.0 or increasing it past 7.0, a drop within the fluorescence intensity of AMF reagent happened leading to diminish within the predictable quenching by the addition of drug.

3.2.4. Effect of the reaction time

The impact of time on the quenching of the fluorescence of AMF by IXA was considered by calculating the reactions each 5 min for 45 min. The impact of reaction time on the fluorescence quenching was shown in (Fig. 9). The results shown that the overall reaction and consequently the greatest senstivity was achieved after 10 ± 2 min, past which there were nearly slight changes within the measured fluorescence.

3.2.5. Effect of dilution solvent

The impact of various weakening solvents was followed after the same approach. Various solvents of different polarities were attempted counting: chloroform, isopropanol, methanol, dimethylformamide (DMF) and refined water. It was found that the chief solvents to be utilized for achieving highest sensitivity at 530 nm was methanol.Typically due to the low energy gap among methanol vibrational energy levels related to water, so senstivity in case of methanol is greater [22].

3.3. Validation of the proposed spectrofluorimetric method

The established method has been validated according to ICH guidelines [23]. All validation parameters are shown in Tables 3–5.

3.3.1. Linearity and range

The linearity of the proposed approach was built up beneath the already optimized conditions employing a set of solutions of various concentrations. A calibration curve (Fig. 10) was built to show the relationship of the fluorescence contrast between the signals of blank solutions of AMF and those achieved after reaction of IXA to the comparing drug concentrations in ng mL−1 which was found to be direct within the area of (20–100 ng mL−1). Regression analysis was achieved by least squares analysis of the calibration results to determine the relation coefficient (r), slope (b), intercept (a), standard deviation of slope (Sb) and standard deviation of intercept (Sa) (Table 3). Test data confirmed acceptable linearity of the proposed approach as shown by the high relationship coefficient ($r > 0.9997$), % RSD of the slope ($Sb\% < 2\%$) and the small value of significance F that shown a small grade of emprical points diffusing around the regression line.

3.3.2. Limit of detection (LOD) and limit of quantitation (LOQ)

LOD is considered as the concentration which can be spoken to by 3 S/m and LOQ by 10 S/m, where, S is the standard deviation and m is the slope of the calibration line. The little values of LOD and LOQ displayed in (Table 3) affirmed the sensible sensitivity of the proposed approach in qualitative and quantitative analysis of IXA.

3.3.3. Accuracy and precision

To evaluate the reliability and repeatability of the proposed approach, the precision and accuracy of estimations have been assessed as beneath the main method. Three readings at each concentration level were done (Table 4). Recovery % and RSD % were determined for each level. The resultes were inside the satisfactory limits of 98–103% and 2% for recoveries and RSD% separately. The intra-day and inter-day precision were evaluated utilizing concentrations inside the linearity area, on the same day and on distinctive days individually. The little RSD % shown the great precision of the proposed approach (Table 4) and affirmed the reliability of the approach for quality control tests of IXA.

3.3.4. Robustness

To test the robustness of the recommended spectrofluorimetric approach, the already detailed approach was performed beneath little varieconnects within the optimized parameters such as volume of AMF reagent solution (± 0.2 mL) and the reaction time (± 2 min). Low RSD% values appeared in (Table 5) affrmed that little varieties within the previously detailed had no critical impact on the analysis of IXA by the recommended approach.

3.4. Stability

3.4.1. Stability of IXA and AMF stock solutions

Two series of solutions of IXA and AMF were set-up and one was stored at room temperature whereas the other was stored in a fridge. The solutions were evaluated each hour for the early 12 h and after that each 24 h for 14 days. Resultes uncovered that the solutions were steady at room temperature for one week and in fridge for 10 days.

3.4.2. Stability of the final ready for measuring reaction solutions

The stability of the latest solutions prepared for measuring their reaction was inspected for one hour at room temperature. It was established that the fluorescence intensity increased drastically after clearing out the test solutions to stand at room temperature for 10 min prior estimations at that point persisted nearly consistent for one hour.

3.5. Analytical applications

3.5.1. pharmaceutical preparation

The proposed approach was practiced for the assurance of IXA in Ixempra® vials. The resultes achieved are appeared in (Table 6). Recovery was achieved by applying the standard addition technique where various concentrations of standard IXA solution (40-80 ng) were included to already analyzed Ixempra® vials. % Recoveries were achieved and are displayed in Table 6. There was no obstructions from co-formulated excipients. Statistical analysis of the resultes achieved by the proposed method and those achieved by the reported approach [6] was done utilizing the student's t-test and the variance ratio F-test (Table 7). The calculated values didn't pass the hypothetical ones showing no significant difference between the proposed approach and the reported one with respect to precision and accuracy.

3.5.2. In plasma

The great sensitivity of the proposed spectrofluorimetric approach permitted the analysis of IXA drug in spiked human plasma. To defeat lattice interferences, tests were subjected to a clean-up method. In this regard, acetonitrile was utilized for protein precipitation. Three concentrations were spiked for the drug and spiked concentration was reproduced three times to affirm the accuracy and precision of the proposed approach. The recoveries were calculated and they diversed between 95–97% (Table 8). Appropriately, this work about spiked plasma tests propose that the proposed approach is performed for the in vivo test of the drug in real biological samples.

4. Conclusion

In this work a solid, fast, taken a toll successful and simple spectrofluorimetric approach was created for the determination of IXA in bulk as well as in Ixempra® vials and human plasma. The approach depends on the measured fluorescence quenching of AMF due to the presence of the sulfide moiety in IXA structure. The approach was statistically validated with regard to precision, accuracy, linearity, area, LOD, LOQ and robustness. All parameters were established to be inside satisfactory limits. Linearity and area were found to be greatly specific as they gave satisfactory recoveries and the correlation coefficient (r) was 0.9998. In addition, it is sensibly delicate and reasonable for dependable investigation of low concentrations of the drug. Both inter-day and intra-day precisions were considered. Resultes of this experiment were found to be inside satisfactory. Subsequently, the proposed spectrofluorimetric approach can be suggested to consider the pharmacokinetics of the drug in numerous preparations and combinations and human plasma.

Data Availability Data available on request

Compliance with Ethical Standards

Conflicts of Interest/Competing Interests The authors declare that they have noconflict of interest.

Ethics Approval Not applicable

Consent to Participate Not applicable

Code Availability Not applicable

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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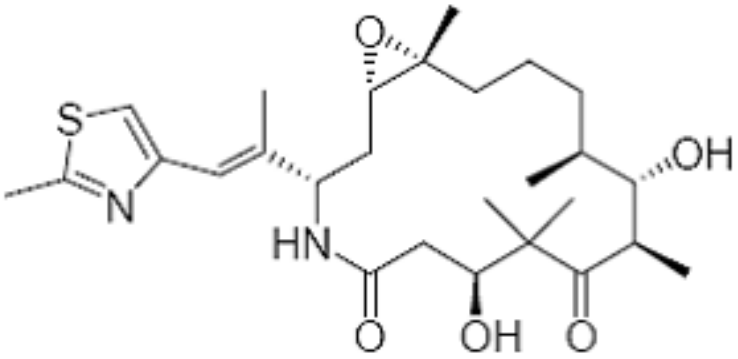


Figure 1. Chemical structure of ixabepilone

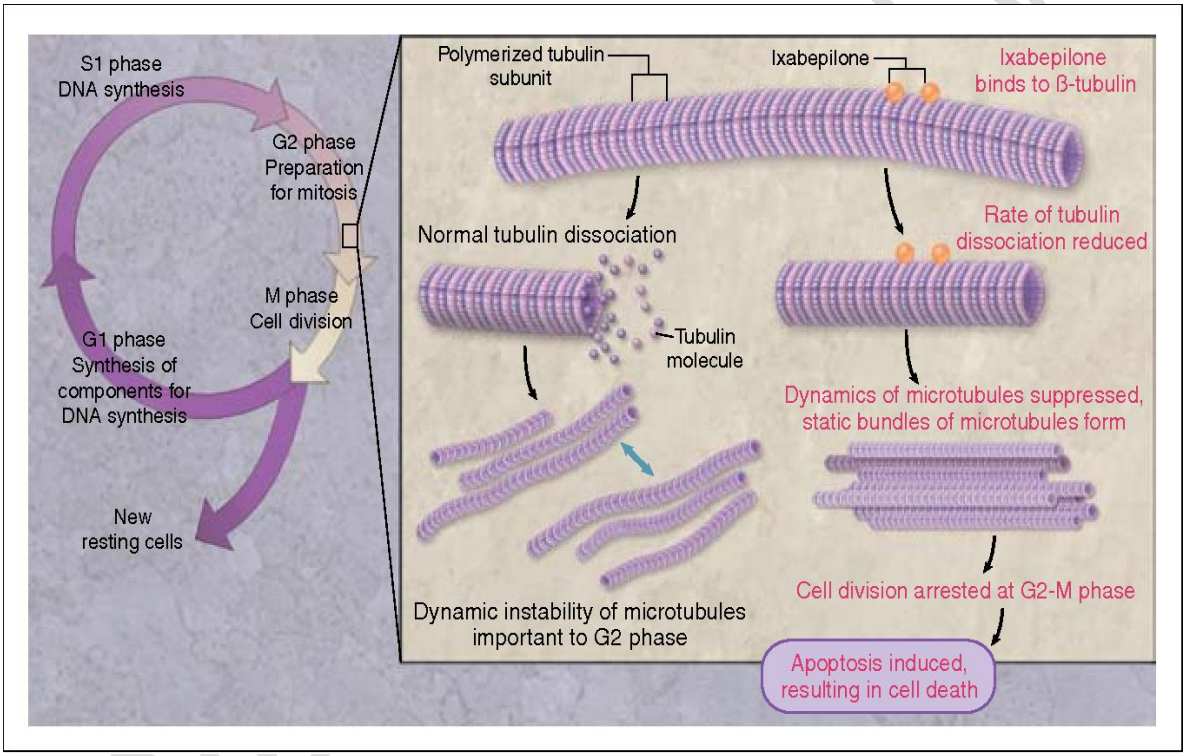


Figure 2. Mechanism of action of ixabepilone. Ixabepilone binds to the b-tubulin subunits of microtubules to induce microtubule polymerization and stabilization, which lead to G2-M arrest and the induction of apoptosis.

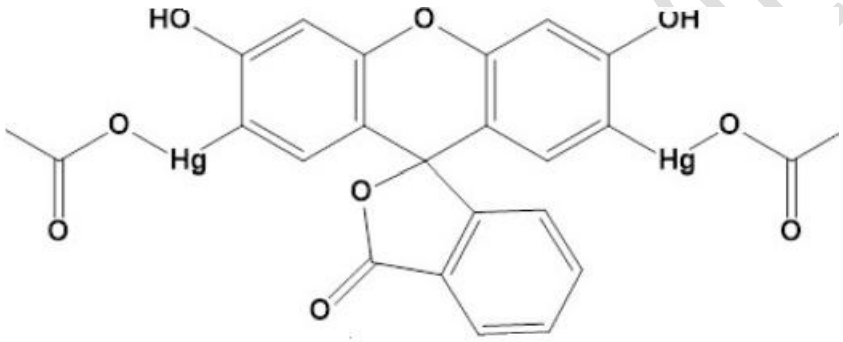


Figure 3. Chemical structure of Acetoxymercuric fluorescein reagent.

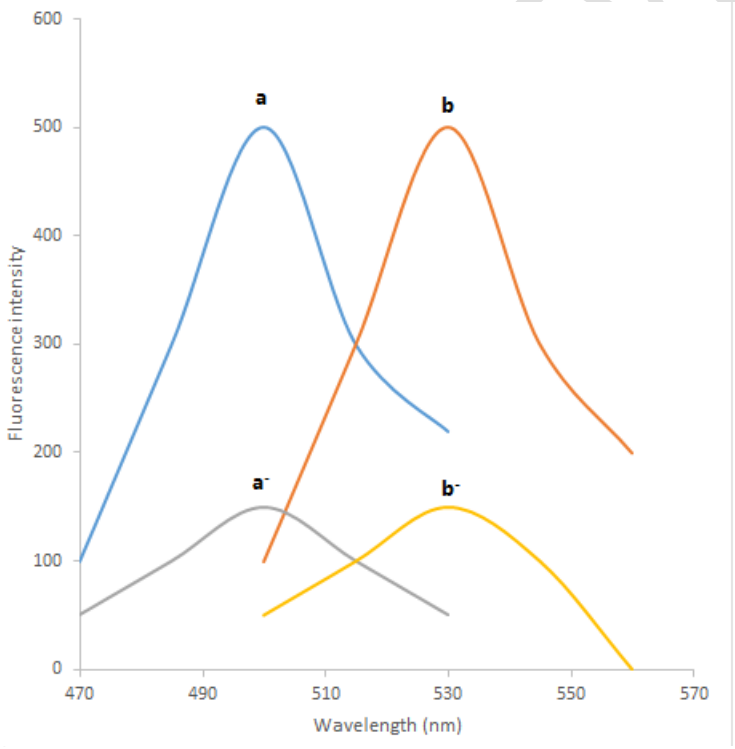


Figure 4. Excitation and emission spectra of 1.0 mL (10^{-4} M AMF solution) in the absence and presence of (120 ng mL^{-1}) IXA at 500 and 530 nm, respectively. a: Excitation blank of AMF, a^{-}: Excitation of AMF+IXA, b: Emission blank of AMF and b^{-}: Emission of AMF+IXA.

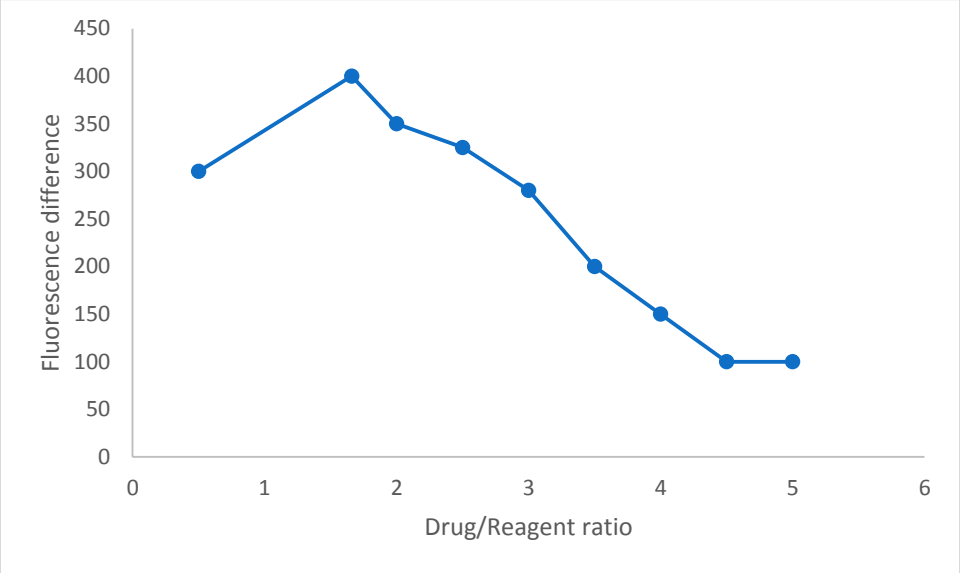


Figure 5. Stoichiometry of the reaction of IXA ($1x10^{-4}$ M) and $1x10^{-4}$ M) AMF by continuous variation (Job's) method.

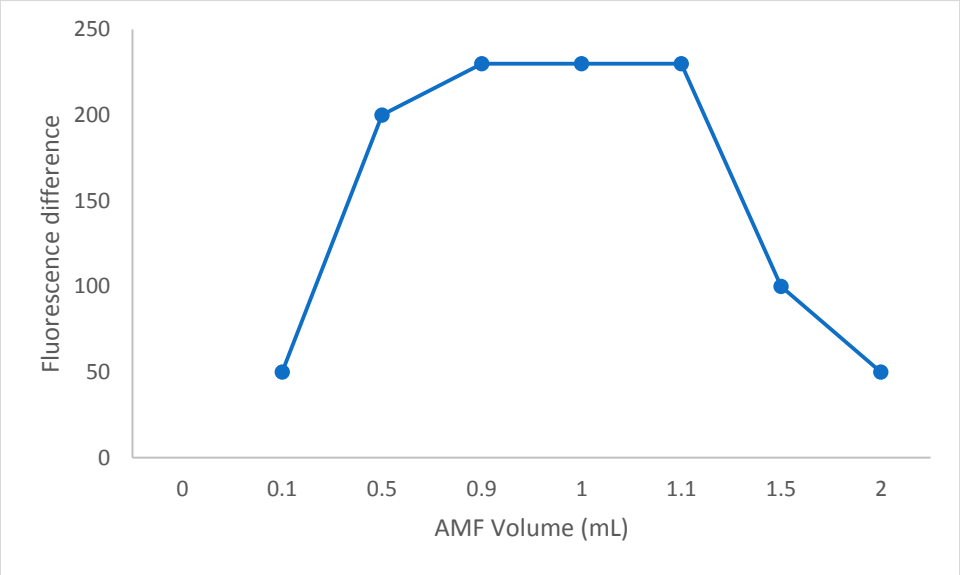


Figure 6. Effect of AMF volume on the fluorescence difference, after the reaction with 60 ng mL^{-1} IXA at 530 nm.

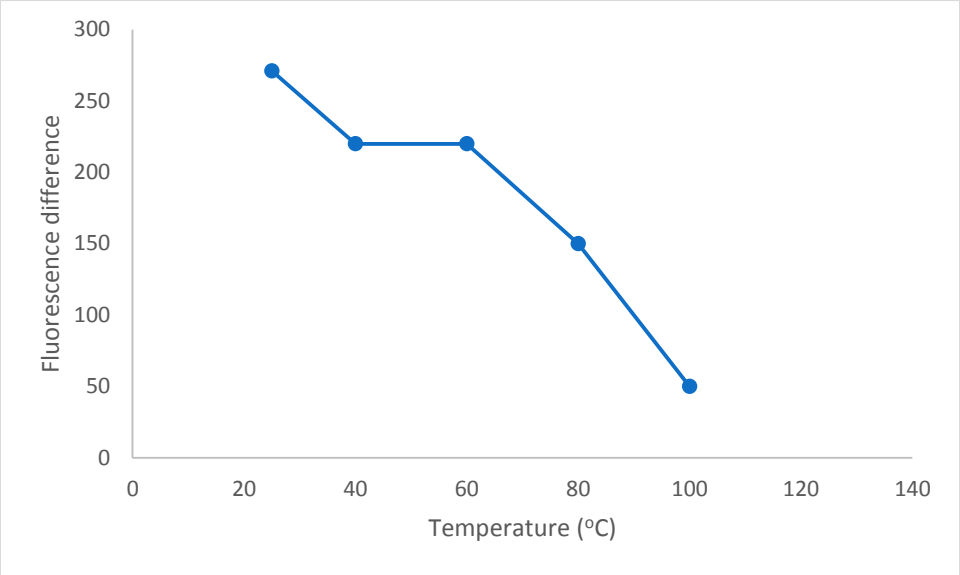


Figure 7. Effect of temperature on the fluorescence quenching 1 mL AMF after the reaction with 60 ng mL^{-1} IXA at 530 nm.

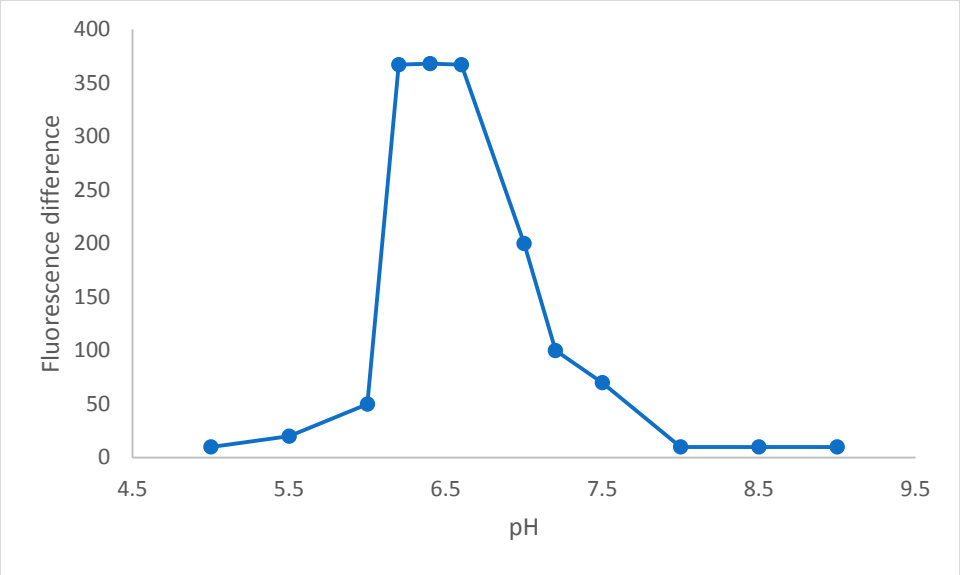


Figure 8. Effect of medium pH on the fluorescence quenching of 1 mL AMF after reaction with 60 nm mL^{-1} IXA at 530 nm.

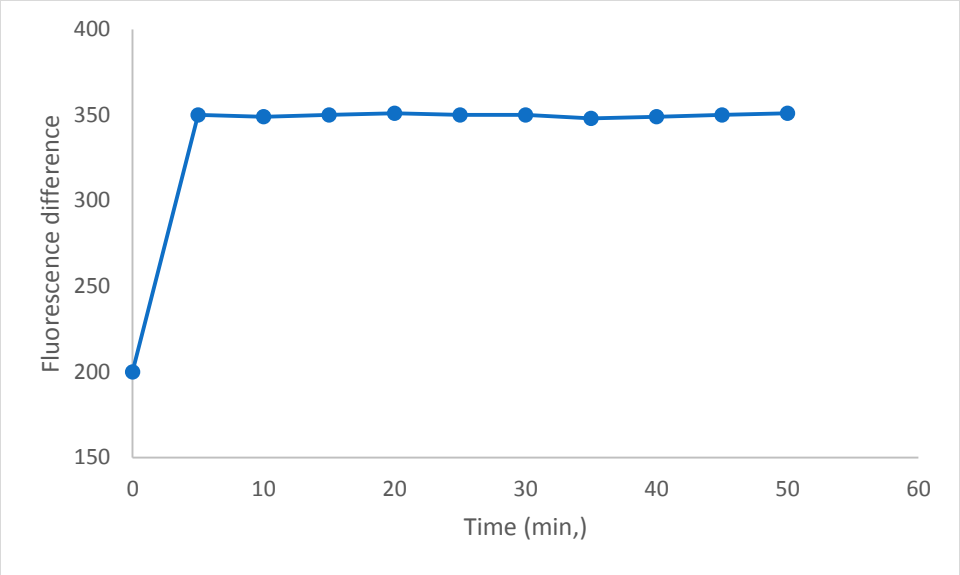


Figure 9. Effect of reaction time on the fluorescence quenching of 1 mL AMF after the reaction with 60 ng mL^{-1} IXA at 530 nm.

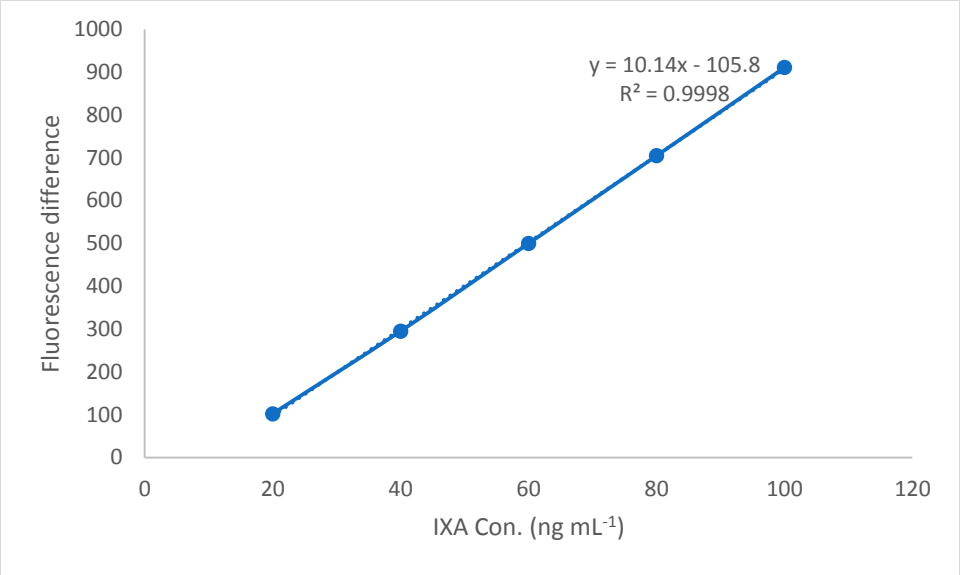
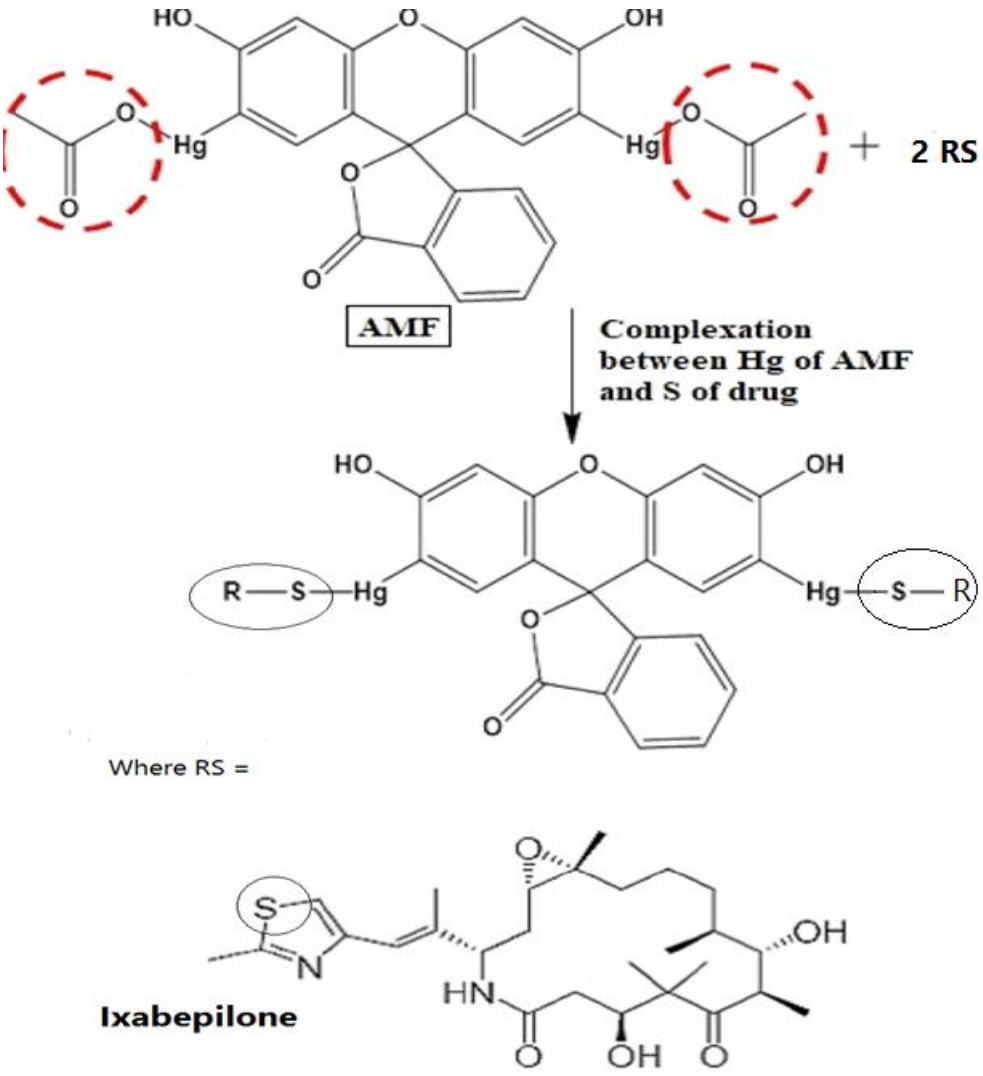


Figure 10. Calibration graph of IXA with $1x10^{-4}$ M AMF at λem 530 nm.



Scheme 1. The proposed mechanism of the reaction between IXA and AMF.

Table 1

Detailed data of the optimization of the reaction parameters.

AMF Con. (µg mL^{-1})	Fluorescence Difference	pH	Fluorescence Difference	Time (min.)	Fluorescence Difference	Temp. (^{o}C)	Fluorescence Difference	Diluting solvent	Fluorescence Difference
0.1	65.689	5	18.6	0	207.987				
0.5	215.564	6	47.387	5	352.123			Water	220.699
1	**240.30**	**6.4**	**366.211**	**10**	**357.254**	**25**	**270.689**	**Methanol**	**260.898**
1.5	115.958	7	116.057	15	352.68	40	235.865	Chloroform	15.785
2	70.032	8	22.288	20	356.68	60	237.868	DMF	17.789
		9	27.998	25	355.964	80	188.547		
				30	351.871	100	59.98		
				35	355.329				
				40	351.985				
				45	354.259				

Mean of (n = 3) experiments for each parameter

Table 2

Assay parameters and conditions for determination of IXA by the proposed spectrofluorimetric method.

Parameters	Proposed method
AMF concentration	$1x10^{-4}$ M
AMF volume	1.0 mL
Temperature	25 ^{o}C
Time	10 min
Diluting solvent	Methanol
pH	6.4

Table 3

Regression parameters and test results for the determination of IXA by the proposed spectrofluorimetric procedure.

Parameters	Spectral data
λex & λem (nm)	500 & 530
Linearity range (ng mL^{-1})	20-100
LOD (ng mL^{-1})	5.145
LOQ (ng mL^{-1})	16.987
Slope ± Sb	10.14± 0.99
Intercept ± Sb	-105.81± 2.67
%RSD of Sb	1.39
Regression equation	Intensity 530 = 10.14 – 105.81
Significance F	5.84×10^{-6}
Correlation coefficient (r)	0.9998

Table 4

Intra-day and inter-day accuracy and precision for the determination of IXA by the proposed spectrofluorimetric method.

IXA ($ng\ mL^{-1}$)	Intra-day			Inter-day		
	Found ±SD ($ng\ mL^{-1}$)	Accuracy (%)	Precision (%RSD)	Found ±SD ($ng\ mL^{-1}$)	Accuracy (%)	Precision (%RSD)
40	40.58±1.06	101.45	1.045	40.82±0.75	102.05	0.735
60	59.66±0.99	99.43	0.996	59.38±0.88	98.96	0.889
80	79.23±0.89	99.04	0.899	80.63±0.69	100.79	0.685

Table 5

Robustness of the proposed method for the determination of IXA

Parameters	%RSD
AMF Volume (±0.2 mL)	1.04
Reaction time (± 2 min)	0.84

Mean of % RSD

Table 6

Recovery of IXA by applying standard addition technique

Ixempra® vials ($ng\ mL^{-1}$)	Drug added ($ng\ mL^{-1}$)	Drug found ($ng\ mL^{-1}$)	% Recovery
80	40	40.74	101.84
80	60	60.34	100.56
80	80	81.74	102.18
80	100	98.49	98.49
Mean			100.77
RSD %			1.85

Table 7

Statistical analysis of the results obtained by the proposed and reported procedures for the determination of IXA in Ixempra® vials

Parameters	Ixempra® vials	
	Proposed method	Reported method [6]
N[a]	5	5
Recovery %	101.62	100.94
SD	0.739	0.939
RSD%	0.716	0.937
t[b] (2.262)	1.27	
F value [b] (5.05)	1.615	

[a] Number of experiments.

[b] The values in parenthesis are tabulated of t and F at (P = 0.05)

Table 8

Statistical analysis of the results obtained by the proposed and reported procedures for the determination of IXA in human plasma

Parameters	IXA	
	Proposed method	Reported method [6]
N[a]	5	5
Recovery %	99.79	100.94
SD	1.597	0.939
RSD%	1.643	0.937
t[b] (2.262)	1.676	
F value [b] (5.05)	2.898	

[a] Number of experiments.

[b] The values in parenthesis are tabulated of t and F at (P = 0.05)