# A green spectrofluorimetric approach for the versatile determination of Bilastine in real human plasma and pharmaceutical preparations with content uniformity testing

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# Abstract

A green spectrofluorimetric method for determining Bilastine (BIL) was described. The method is very sensitive, simple, and quick. The suggested method was depend on the measurement of the original fluorescence of BIL in 1.0 M sulfuric acid at an emission wavelength of 385 nm after an excitation at 272 nm. The method was evaluated by ICH requirements. The relationship between BIL concentrations and the fluorescence intensities was linear in a range of 10.0–500.0 ng $mL^{-1}$, and the correlation coefficient was 0.9999. The detection limit was 2.9 ng $mL^{-1}$ and quantitation limit was 8.8 ng $mL^{-1}$. The suitable sensitivity and selectivity of the suggested method enabled its application successfully in analyzing BIL in real human plasma with appropriate recoveries from 95.72% to 97.2 %, as well as in pharmaceutical preparations without any interfering effect from plasma components or pharmaceutical excipients. Additionally, the suggested method was utilized efficiently for content uniformity test.


# 1. Introduction

Bilastine (BIL) is a new second-generation antihistaminic drug that is taken orally to treat the symptoms of urticaria and rhino-conjunctivitis, which can be seasonal or chronic [1]. The European Medicines Agency (EMA) approved BIL in September 2010 [2]. The recommended dose of BIL is 20 mg once daily for the management of urticaria and rhino-conjunctivitis symptoms. BIL has a high affinity for $H_1$ receptors and has slight or no affinity for other receptors, including some histamine receptor subtypes, muscarinic and 5-HT receptors. So, it has no central nervous system (CNS) effects. BIL has a 6 and 3 fold higher affinity than fexofenadine and cetirizine, respectively [1, 3]. The chemical structure of BIL is; 2-[4-(2-(4-(1-(2-ethoxyethyl)-1H-benzimidazol-2-yl)piperidin-1-yl)ethyl)phenyl]-2-methylpropionic acid (Fig. 1) [4]. The available literature review revealed a variety of methods for analyzing BIL in bulk or pharmaceutical formulations. The methods that have been published for the analysis of BIL were spectrophotometric [5–7], fluorometric [8], HPLC [9–12], hydrophilic interaction liquid chromatographic method [4], HPTLC [13], Near-infrared spectroscopy [14], and electrochemical methods [15]. The HPLC technique needs a lot of extremely pure organic solvents, takes a long time to prepare samples, uses complicated apparatus, and, in certain situations, requires very expensive detectors. Furthermore, the sensitivity of spectrophotometric methods is limited yo be applied in biological fluids analysis.

Although direct spectrofluorimetric techniques have a great sensitivity, selectivity, and inherent simplicity [16, 17], only one method has been published for assaying BIL [8]. Owing to its rigid structure, BIL exhibits a native fluorescence. However, the excitation and emission wavelengths of BIL in aqueous medium were 272 and 298, respectively. Hence the stock shift is very small (≈ 26 nm), which resulted in high inner filter effect. The inner filter effect impedes fluorescence measurements by restricting fluorescence signal linear dependence to narrow concentration rang [18, 19]. On the other hand, using sulfuric acid as a media for BIL, shifted the excitation and emission wavelengths to higher wavelengths (272 and 385 nm respectively) with higher stock shift (≈ 103 nm). As a result, the inner filter effect was effectively diminished and wide concentration range could be determined. The suggested method was found to be

compliant with the International Council on Harmonization (ICH) requirements [20], and it was successful in detecting BIL in bulk forms, and pharmaceutical formulations. Furthermore, the method was extended to determine BIL in real human plasma and to examine tablet content uniformity. The proposed method includes the use of water as a solvent which is the most eco-friendly solvent in analytical chemistry. In addition, the proposed method has the following advantages: short analysis time, lower financial expense and high simplicity.

## 2. Experimental

## 2.1. Instrumentation

The spectrofluorimetric measurements were performed with JASCO FP-8350 spectrofluorometer (Hachioji, Tokyo, Japan). The instrument has a 150 W Xe-arc lamp and a PMT adjusted to a voltage of 400 V. Slits width for both emission and excitation monochromators were 5 nm, and the scanning rate was 1000 nm per min. Distilled water was obtained using Aquatron water still a4000d (Cole-Parmer, Staffordshire, UK).

## 2.2. Materials and reagents

BIL and Contrahistadin® tablets containing 20 mg of BIL (B.N. H01702), were obtained from Global Advanced Pharmaceuticals (6th of October, Egypt). Spectroscopic grade methanol, ethanol, acetonitrile, tween, β-cyclodextrin (β-CD), sodium carboxymethyl cellulose (CMC Na), polyvinyl alcohol (PVA), citric acid, and perchloric acid were supplied from Merck (Darmstadt, Germany). Analytical grade dimethylformamide (DMF), sodium hydroxide, polyethylene glycol 400 (PEG 400), PEG 6000 were supplied from Fischer Scientific (Loughborough, U.K). Analytical grade acetone, sodium dodecyl sulfate (SDS), acetic, hydrochloric, sulfuric, nitric, phosphoric acid were supplied by El Nasr Pharmaceutical Chemical Co. (Cairo, Egypt). Boric, citric acid, and phosphoric acid sodium hydroxide were utilized for the preparation of Teorell - Stenhagen buffer solution pH (3–10) [21].

## 2.3. Preparation of standard solution

Ten milligrams of BIL were dissolved in 250 mL distilled water to make stock standard drug solutions (40 μg $mL^{-1}$). A portion of standard solution was diluted with distilled water to get working standard solutions that were used for calibration curve establishment.

## 2.4. Procedures for general assay

Standard solutions of BIL in concentrations ranging from 0.1 to 5 μg $mL^{-1}$ were transferred into 10 mL volumetric flasks, then 2 mL of 1 M sulfuric acid was added. After that, the flasks were filled to the final volume using distilled water and the contents were mixed thoroughly. The intensities of the fluorescence of the resulting solutions were monitored at 385 nm ($\lambda_{ex}$ at 272 nm). A blank was processed using the previous steps except adding the BIL solution. Plotting the obtained values of the fluorescence versus the concentrations of BIL was carried out for construction of the calibration plot.

## 2.5. Procedure for the analysis of BIL in tablets formulation

Ten Contrahistadin® 20 mg tablets were precisely weighed and finely powdered. A portion of the fine powder containing 10.0 mg of BIL was moved to a 100 mL volumetric flask containing 30 mL of double-distilled water and the content was sonicated for 30 minutes. The flask was completed to the final volume with distilled water to get a solution of 100 µg $mL^{-1}$ of BIL. After filtration and removing the initial part of the filtrate, then aliquot was analyzed by applying the general analytical procedure in five duplicates.

## 2.6. Procedure for content uniformity test

The content uniformity (CU) test for BIL in tablet formulation was performed in accordance with USP requirements (Chap. 905) [22]. A separate analysis of 10 Contrahistadin® 20 mg tablets was used for testing the uniformity of their contents using the procedure described under the analysis of pharmaceutical tablets for each individual tablet.

## 2.7. Procedure for estimation of BIL in spiked human plasma

Samples of human blood were obtained from healthy volunteers at the Hospital of Sohag University and collected into a series of heparinized tubes. Each volunteer was informed about the goal of the experiment and gave his written consent. The use of plasma samples from humans was agreed with the Declaration of Helsinki Recommendations [23]. To separate plasma proteins, centrifugation of 5 ml of the blood sample was performed at 4000 rpm for 30 minutes. The obtained plasma was placed in Eppendorf's tubes and kept at -20 $^{o}$C. Into a clean tube, 1.0 ml of the stored plasma was transferred, 1.0 ml of BIL standard solution (final concentrations of 0.1–5 µg $mL^{-1}$) was added, then an appropriate volume of acetonitrile was added as proteins precipitating agent. The tube was mixed by vortex for 60 seconds before being centrifuged for 10 minutes at 4000 rpm. [24]. The clear supernatant was moved to a clean tube and the general method procedure was followed. A blank experiment was treated similarly using distilled water instead of the standard drug solution.

## 2.7. Procedures for estimation of BIL in real human plasma

Five healthy volunteers (Sohag University Hospitals) were given a single dose of BIL (Contrahistadin® 20 mg/tablet) and blood samples were drained from them after 1.3–1.4 hours. A total of 5.0 mL of volunteer's blood was collected into a series of tubes containing heparin and centrifuged at 4000 rpm for 30 minutes to separate the plasma. Finally, the plasma solutions were subjected to the proposed analytical procedure.

# 3. Results And Discussion

The high fluorescence intensity of BIL is due to the presence of the benzimidazole ring in its structure. The excitation and emission wavelengths of BIL in aqueous medium were 272 and 298, respectively with

a very small stock shift (≈ 26 nm). Accordingly, the inner filter effect would be very high. The inner filter effect impedes fluorescence measurements by reducing the linear dynamic rang of the method [18, 19]. However, by examining the fluorescence characteristics of BIL in sulfuric acid media, it was found that BIL has strong native fluorescence at 385 nm (excitation at 272 nm), Fig. 2 which, have higher stock shift and lowered inner filter effect than that in pure water.

# 3.1. Optimization of the experimental condition

The effects of various experimental conditions on the fluorescence intensity of BIL were tested, and the best parameters for achieving the maximum fluorescence intensity were determined.

# 3.1.1. Effect of buffers and pH modifier

To enhance the native fluorescence of BIL, 1.0 ml Teorell-Stenhagen buffer solutions of varying pH (3.0–10.0) were examined in addition to 1.0 ml of 1 M $H_2SO_4$ or NaOH, Fig. S1. The best fluorescence intensity of BIL was obtained with 1 M $H_2SO_4$. Furthermore, it decreased the IFE. Different types of acids (1.0 M) were also investigated namely, sulfuric, perchloric, acetic, hydrochloric, phosphoric, and nitric acids, Fig. 3. The highest fluorescence intensity was obtained with 1 M sulfuric acid, so, it was used in the subsequent work as the pH modifier. Finally, the effect of 1 M sulfuric acid volume was examined in the range from 0.5–5 mL, Fig. 4. The highest fluorescence intensity was obtained with the use of 2 mL of 1 M sulfuric acid.

# 3.1.2. Effect of different organized medium

Various organized media were utilized in the study to enhance the fluorescence of the aqueous BIL solution. Anionic surfactant (SDS, 0.288% w/v), nonionic surfactant (PEG 6000, 1% w/v, PEG 400, 1% v/v and tween 80, 1% v/v, PVA, 1% w/v), anionic polysaccharide (CMCNa, 1% w/v) and macromolecules (β-CD, 1% w/v) were studied (Fig. S2). It was observed that; the studied substances did not enhance the fluorescence intensity of BIL; indeed, tween 80 significantly reduced the drug's native fluorescence intensity. As a result, no organized medium was used.

# 3.1.3. Effect of diluting solvent

Water, acetone, ethanol, methanol, dimethylformamide, and acetonitrile were studied to dilute BIL, Fig. 5. The greatest fluorescence intensity was obtained when water was the diluting solvent. The use of water is of a great advantage for the present work since water is eco-friendly, inexpensive, and readily available.

# 3.2. Methods validation

ICH guidelines [20] were applied to evaluate and validate the proposed native fluorescence method.

### Linearity and range

The calibration curve was constructed by plotting various standard solution concentrations of BIL versus the fluorescence intensity. Linearity was achieved for the current method in concentrations ranging from 10 to 500 ng $mL^{-1}$ and the correlation coefficient was 0.9999, indicating that; the suggested method has excellent linearity. The various analytical parameters were summarized in Table 1.

Table 1
The regression and validation parameters for the proposed method

| Parameter | Proposed Method |
|---|---|
| Linear range (ng $mL^{-1}$) | 10–500 |
| Slope | 13.7764 |
| SD of slope ($S_b$) | 0.0526 |
| Intercept | -43.3058 |
| SD of intercept ($S_a$) | 12.1247 |
| Correlation Coefficient | 0.9999 |
| SD of residuals ($S_{y,x}$) | 27.3334 |
| LOD (ng $mL^{-1}$) | 2.904 |
| LOQ (ng $mL^{-1}$) | 8.801 |

### Limits of detection and quantification

The method's sensitivity was tested using the limits of detection (LOD) and limits of quantification (LOQ) calculations. The LOD and LOQ were estimated by applying the ICH guidelines equations LOD = 3.3 SD/b and LOQ = 10 SD/b (b is the slope and SD is the standard deviation of intercept). The found LOD was 2.9 ng $mL^{-1}$, while the calculated LOQ was 8.8 ng$mL^{-1}$, proving the current method is highly sensitive in the assay of BIL. The aforementioned results propose the ability of the current method to quantify BIL in real human plasma due to it has excellent sensitivity.

### Accuracy and precision

The accuracy of the provided fluorometric procedure has been examined using triplicate measurements of various BIL concentrations and using a standard addition method for each concentration. The obtained results demonstrated that the calculated values were highly agree with the actual values, indicating good precision of the suggested method, Table 2. Using the previously analyzed concentrations, the proposed fluorometric method has been tested for inter-and intra-day assay precision. To confirm intra-day precision, the experiment was repeated three times in one day (repeatability). To evaluate inter-day (intermediate) precision, the examined concentrations were measured across three days. As shown in Table 3, all the relative standard deviation values were below 2%, proving the excellent precision of the method.

Table 2
Accuracy of the proposed method using standard addition method

| Amount taken from Contrahistadin® (ng $mL^{-1}$) | Amount added (ng $mL^{-1}$) | Amount found (ng $mL^{-1}$) | % Recovery ± SD[a] |
|---|---|---|---|
| 50 | 0 | 50.084 | 100.17 ± 0.59 |
| 50 | 50 | 100.121 | 100.12 ± 0.74 |
| 50 | 150 | 197. 897 | 98.95 ± 0.64 |
| 50 | 250 | 300.125 | 100.04 ± 0.42 |

[a]Mean of five determinations

Table 3
Evaluation of the intra-day and inter-day precision for the proposed method.

| Conc. level | % Recovery ± RSD [a] | |
|---|---|---|
| ng $mL^{-1}$ | Intra-day precision | Inter-day precision |
| 100 | 99.98 ± 0.38 | 99.94 ± 1.14 |
| 200 | 99.96 ± 0.61 | 100.19 ± 0.94 |
| 400 | 100.41 ± 0.41 | 100.51 ± 0.96 |

[a] Mean of five determinations

## Robustness

Upon introducing small variations in the parameters of the method, no effect was observed in the performance of the developed method. Fortunately, the method included only one parameter that could be examined, sulfuric acid volume. Minor variations in sulfuric acid volume had no apparent effect on the

efficiency of the method. When 1.5 ml sulfuric acid was added, the recovery ± SD (mean of five determination) were found 98.23 ± 0.44 and When 2.5 ml sulfuric acid was added, the recovery ± SD were found 98.02 ± 0.51. Thus, the proposed fluorometric method was found to be robust.

### Selectivity

The influence of tablet excipients included in tablet manufacturing was explored, and the extent of their interference with the suggested approach was evaluated, to check the selectivity of the current method. Talc, zinc oxide, magnesium stearate, lactose, glucose and starch were tested. The results demonstrated the absence of any interfering effect from the examined excipients on the suggested method, as evidenced by the good recovery shown in Table 4.

Table 4
Evaluation of the selectivity for the proposed method.

| Substance added | Amount added | Drug taken | % Recovery ± SD [a] |
|---|---|---|---|
| | ng $mL^{-1}$ | ng $mL^{-1}$ | |
| Talk | 10000 | 100 | 99.03 ± 0.36 |
| Zinc oxide | 10000 | 100 | 100.60 ± 0.55 |
| Magnesium stearate | 10000 | 100 | 100.70 ± 0.66 |
| Starch | 10000 | 100 | 99.95 ± 0.44 |
| glucose | 10000 | 100 | 100.41 ± 0.48 |
| Lactose | 10000 | 100 | 100.12 ± 0.98 |

[a] Mean of five determination

## 3.3. Pharmaceutical application

The suggested method was suitable for analyzing BIL in pharmaceutical dosage forms (Contrahistadin® tablets). Table 5, shows that the percentage recoveries obtained were satisfactory, indicating that there is no matrix effect. For comparing the obtained results of the current method with the reported method results [5], the F-and student's *t*-tests were used. Because the estimated values of both parameters were smaller than the tabulated values at the 95% confidence level, it was established that the accuracy and precision of the suggested method were not significantly differ from the reported method.

Table 5
Application of the proposed methods for the determination of BIL in Contrahistadin® tablets (n = 5).

| Parameters | Reported method | proposed method |
|---|---|---|
| % Recovery [a] | 99.40 | 99.16 |
| Standard deviation, SD | 1.20 | 0.86 |
| Number of determinations | 5 | 5 |
| t-value [a] | | 0.366 |
| F-value [a] | | 1.957 |

[a] Tabulated value at 95% confidence limit; t = 2.306 and F = 6.338.

# 3.4. Application to content uniformity (CU) test

If the proportion of active elements in the tablet formulation units does not go beyond 25% of the entire weight of the tablet or if the content of the active constituent is less than 25 mg, it is advised that the CU of the tablet units should be investigated [22, 25, 26]. For the first time, the spectrofluorometric method was utilized to track the CU of BIL in commercial tablets. Furthermore, the developed method had a very simple analytical process. As a result, the presented spectrofluorimetric method is ideal for this purpose. As presented in Table 6, the acceptance value (AV) was lower than or equivalent to the maximum permissible acceptance value (L1), thus it was concluded that, the active ingredient quantity was uniform in the studied pharmaceutical tablets. The AV could be calculated using the following equation:

Table 6
Application of the proposed methods for the content uniformity test of Contrahistadin® tablets.

| Tablet number | Proposed method |
|---|---|
| 1 | 98.52 |
| 2 | 100.19 |
| 3 | 98.23 |
| 4 | 103.97 |
| 5 | 101.21 |
| 6 | 102.73 |
| 7 | 103.31 |
| 8 | 98.52 |
| 9 | 98.02 |
| 10 | 97.44 |
| Mean $\bar{X}$ | 100.22 |
| S | 2.43 |
| AV* | 6.57 |
| L1* | 15` |

*L1: maximum allowed acceptance value, AV: acceptance value.

$AV = KS + \left| M - \bar{X} \right|$ [26]

where S represents the standard deviation, K represents the acceptability constant, M represents the reference value, and $\bar{X}$ is the mean of each tablet content. The results obtained using the current spectrofluorimetric method for the analysis of Contrahistadin® tablets (20 mg/tablet of BIL) were lesser than the L1 value.

# 3.5. Biological samples application

# 3.5.1. Spiked human plasma application

It was reported that, BIL achieved its maximum plasma concentration ($C_{max}$ = 220 ± 62 ng $mL^{-1}$) 1.3–1.5 hours after oral administration [1, 3, 27]. BIL has a higher plasma protein binding ratio (84–90%) and approximately 95% of BIL was detected unchanged in plasma. BIL is not metabolized to significant extent in humans and is nearly removed from the body unchanged through both urine (33%) and feces

(67%). Because the current method is highly sensitive, it was feasible to estimate BIL in biological fluids. In the analysis of spiked human plasma, the percentage recoveries were in the range of 95.72–97.24%. The results in S3, assured that; the suggested method was suitable for the precise assay of BIL in human plasma with no significant interference related to the matrix.

## 3.5.2. Real human plasma application

Because of its excellent sensitivity, the proposed assay has the ability for the detection of BIL in real human plasma. BIL is measured in real human plasma after administering a single oral dose (20 mg/tablet) of BIL by healthy volunteers. It was reported that the maximum serum concentration ($C_{max}$) of BIL was 220 ± 62 ng $mL^{-1}$ and reached its maximum in 1.35 hours following oral administration. The suggested method was able for the direct in-vivo assessment of BIL in plasma (Table 7) as the $C_{max}$ value is within the linear concentration range of the method (10–500 ng $mL^{-1}$) with LOQ of 8.8 ng $mL^{-1}$. In the present study, the mean plasma level of BIL from five healthy volunteers receiving a single dose of BIL was 227.9 ± 37.3 ng $mL^{-1}$. These values are close to the previously reported one. Meanwhile, the proposed method is characterized by a fast protein precipitation step with acetonitrile, which resulted in a rapid bioanalysis time. Moreover, no sample enrichment steps are required, and no need for repeated extraction steps using toxic organic solvents.

Table 7
Application of the proposed methods for the determination of BIL in real human plasma.

| Human volunteer | Gender (age) | BIL ng $mL^{-1}$ [a] |
|---|---|---|
| 1 | M (27) | 206.23 |
| 2 | F (25) | 264.30 |
| 3 | M (30) | 202.60 |
| 4 | M (42) | 193.89 |
| 5 | M (29) | 272.28 |
| Mean ± SD | | 227.86 ± 37.29 |

F, female; M, male

[a] Mean of five determinations.

# 4. Conclusion

The proposed spectrofluorometric method is very selective to determine BIL tablets' formulations with no interference from their excipients. The presented work has the following advantages: it is sensitive, accurate, and precise when it comes to the analysis of the aforementioned antihistaminic drug in bulk, commercial tablet formulation. The simple procedure of the method enabled its application in tablet content uniformity. It was also able to analyze BIL in spiked and real human plasma containing the drug due to the method's good sensitivity (LOD = 2.9 ng $mL^{-1}$). Furthermore, it is a time-saving method that eliminates the tedious steps for sample preparation or extraction. Due to its simplicity and sensitivity, this method is an excellent candidate for BIL quality control. The use of distilled water as a green solvent makes the proposed procedure a good alternative for conventional methods that use harmful organic solvents.

# Declarations

**Authors' contributions**:

**Sayed M. Derayea**: Conceptualization, supervision, reviewing, and editing. **Khalid M. Badr El-din:** data handling, reviewing and editing. **Ahmed S. Ahmed**: methodology, data manipulation, and writing the first draft. **Ahmed Khorshed:** investigation, validation, methodology. And **Mohamed Oraby**: methodology, validation, reviewing, and writing. All authors reviewed the manuscript.

**Conflicts of interest/Competing interests**

The authors declare they have no competing interests.

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**Ethics Declaration statement**

The research including plasma samples from humans was carried out following the Declaration of Helsinki Recommendations

**Consent to Participate**

Each human volunteer was informed about the goal of the experiment and gave his written consent

**Consent for publication**

Not applicable

**Data availability**

All data generated or analysed during this study are included in this published article and its supplementary information files.

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# Figures

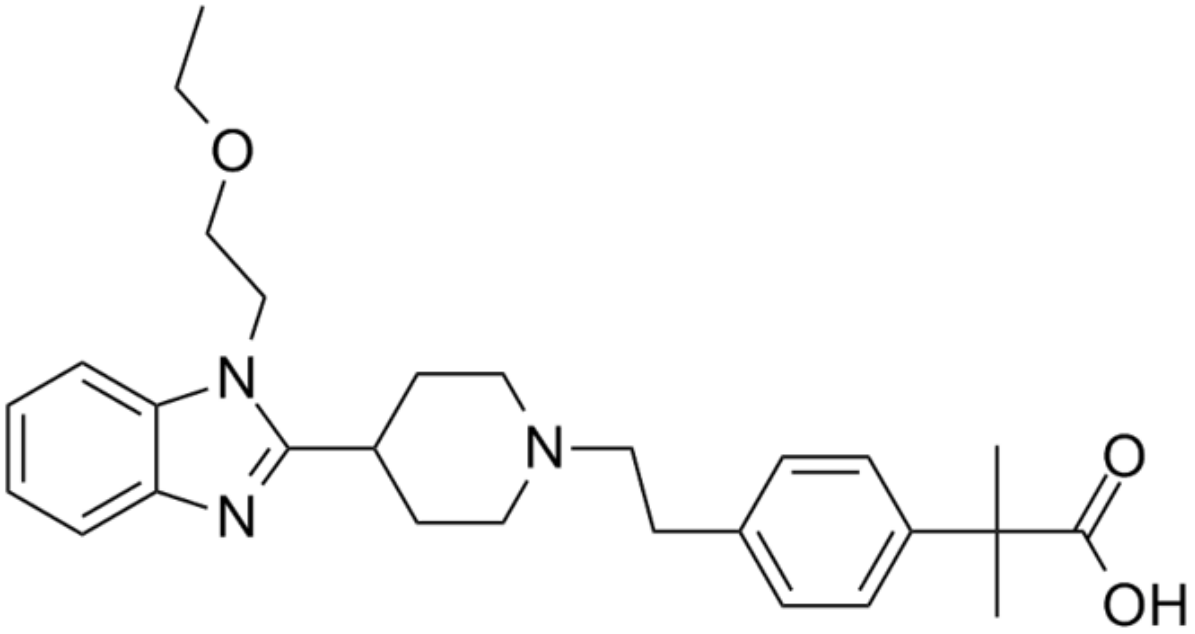



**Figure 1**

The chemical structure of BIL.

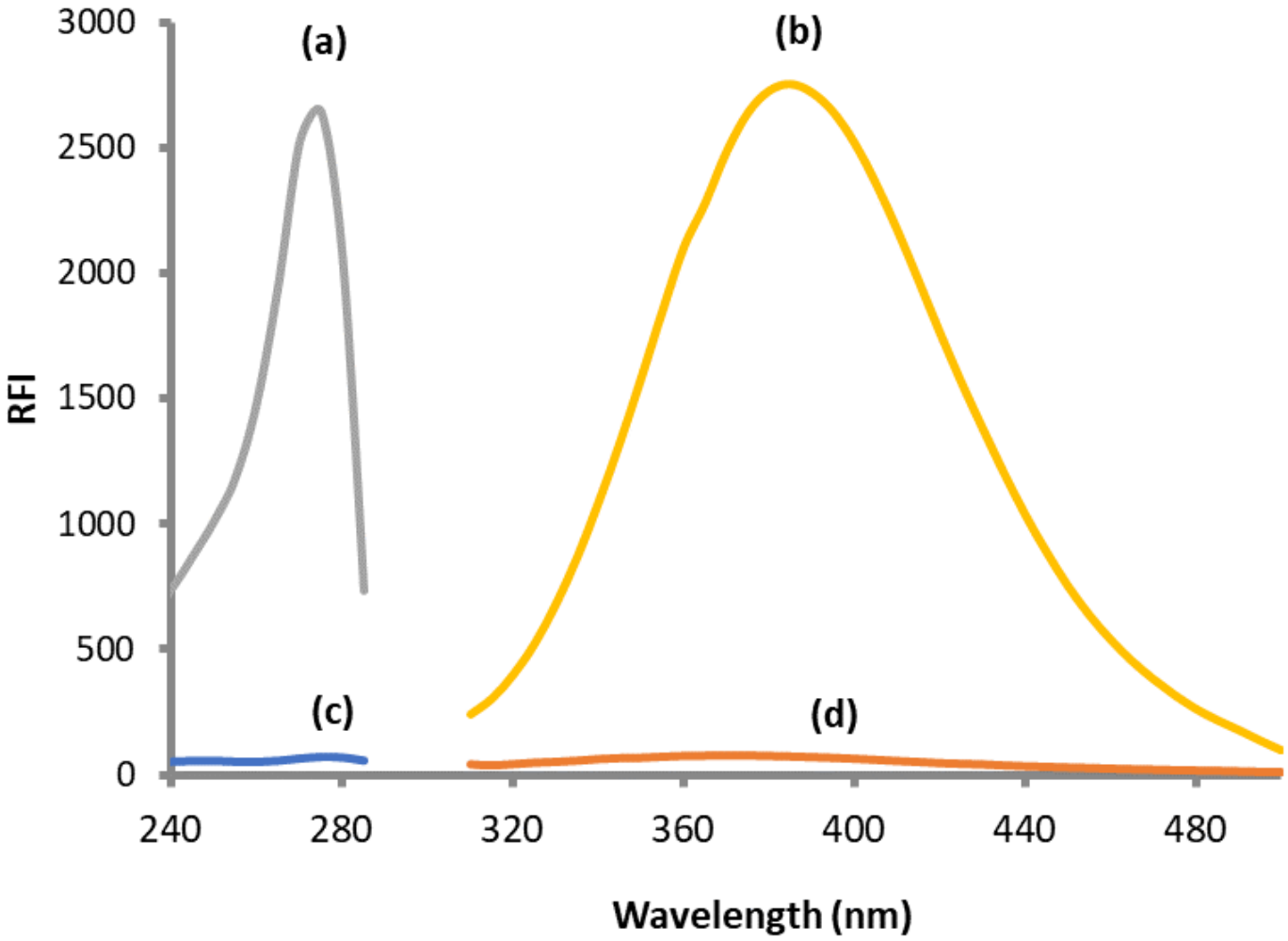
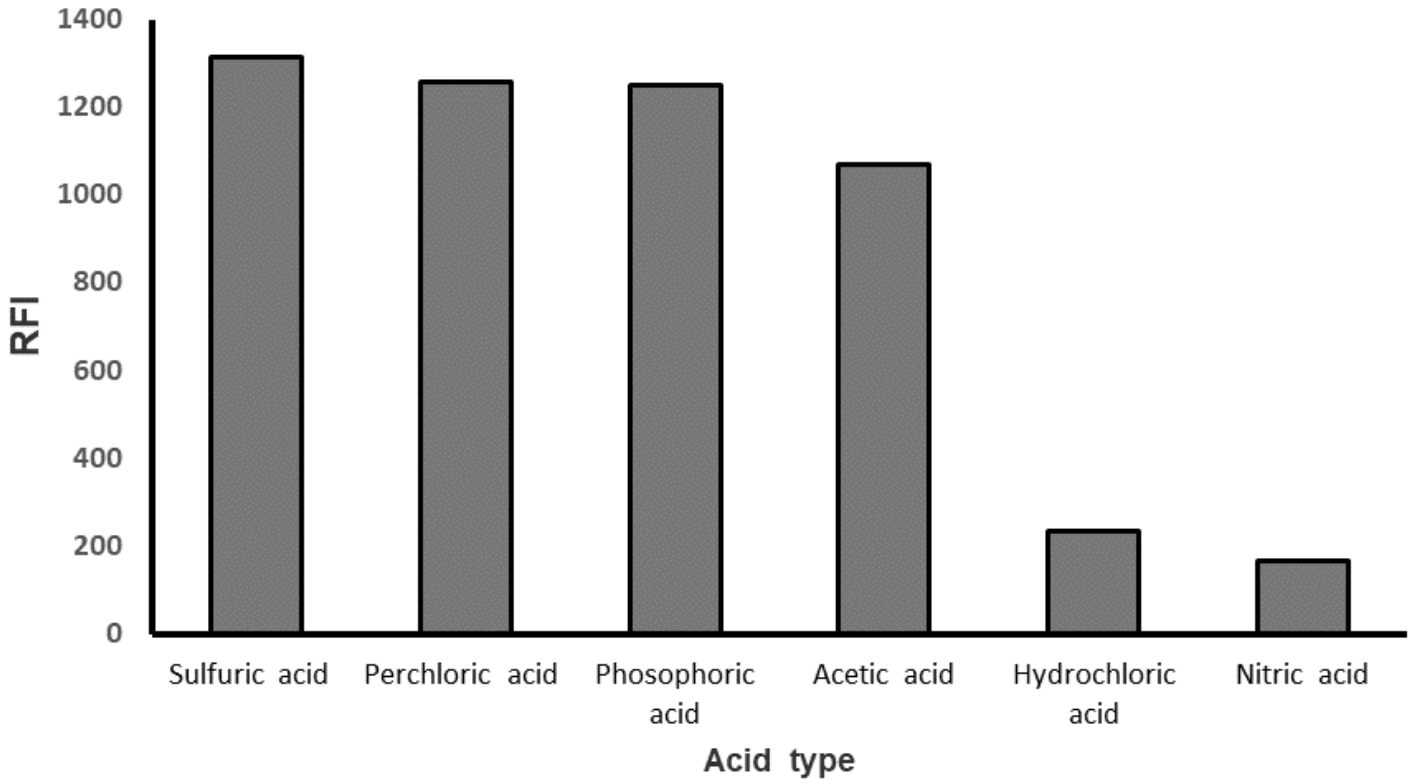


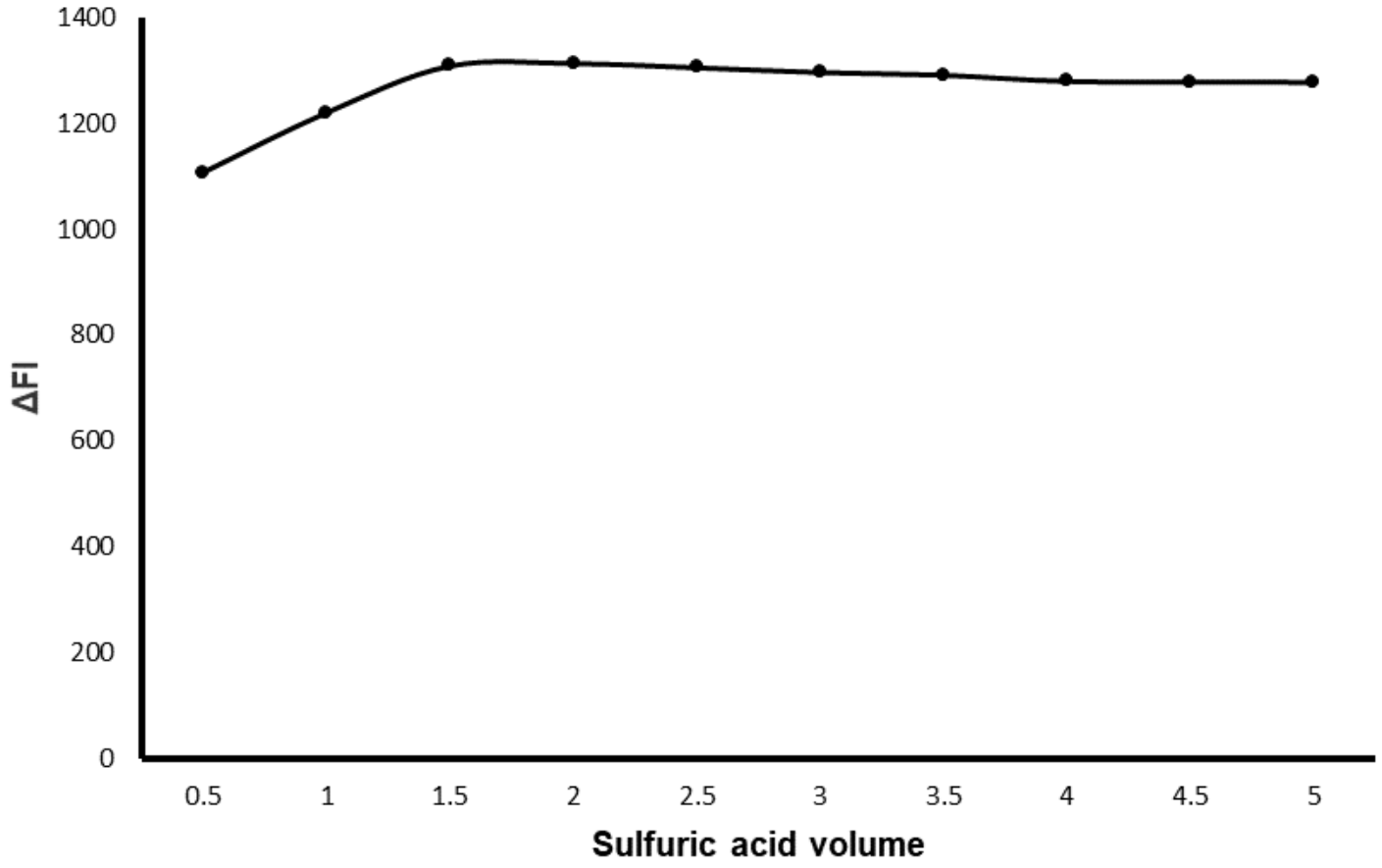

**Figure 2**

Emission (a) and excitation (b) spectra of 200 ng $mL^{-1}$ BIL and the excitation (c) and emission (d) spectra of blank all in sulfuric acid medium.




## Figure 3

Effect of different acids on relative fluorescence intensity of 100 ng $mL^{-1}$BIL.




## Figure 4

Effect of 1 M sulfuric acid volume on relative fluorescence intensity of 100 ng $mL^{-1}$ BIL.

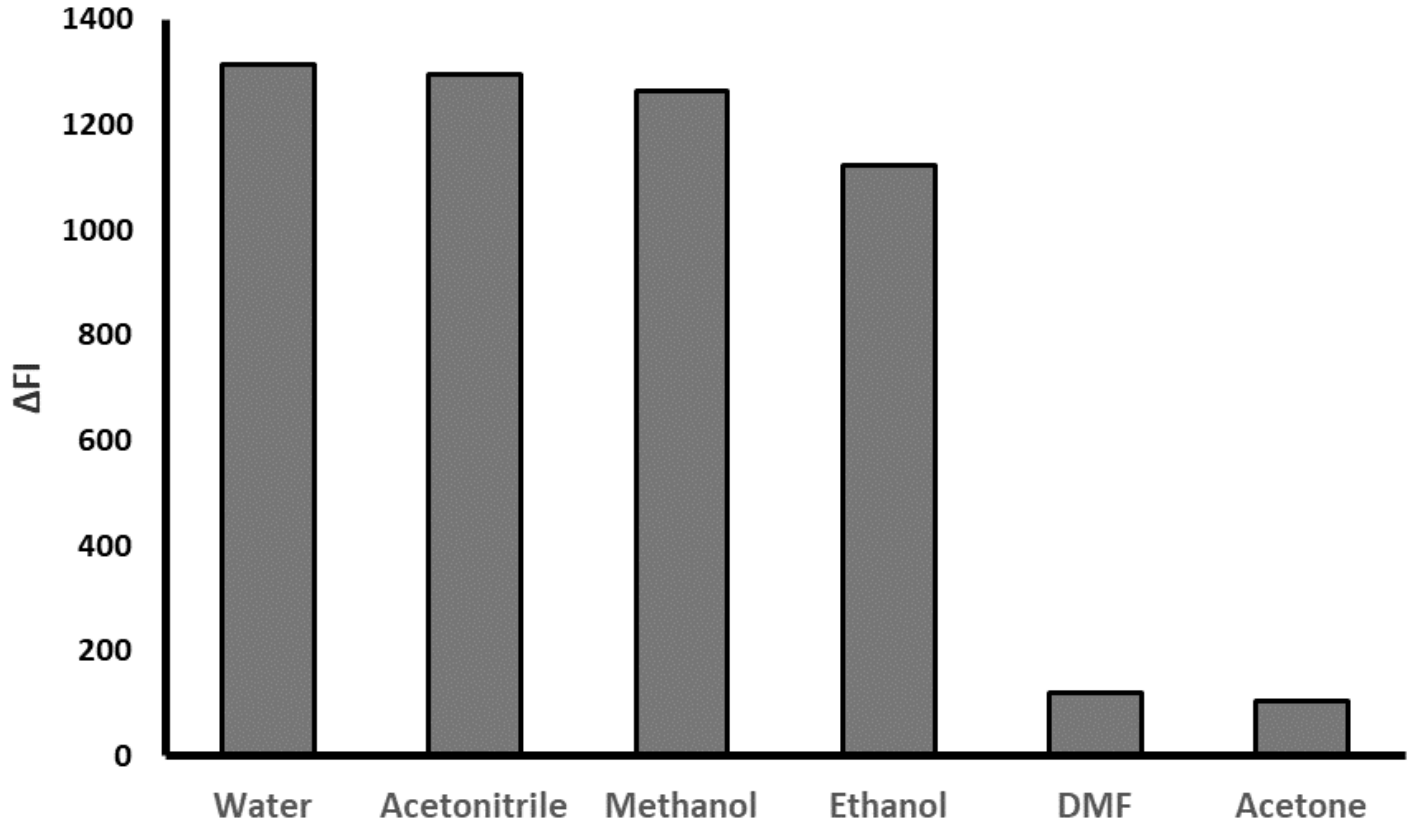



**Figure 5**

Effect of diluting solvent on relative fluorescence intensity of 100 ng $mL^{-1}$BIL.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementarymaterialsBIL.docx