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Development and validation of a spectrofluorophotometric method for berberine hydrochloride quantification in diverse matrices

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ABSTRACT: Berberine hydrochloride (BHC), a key isoquinoline alkaloid from the Berberis genus, is gaining prominence for its diverse pharmacological properties. The goal was to devise rapid and sensitive spectrofluorophotometric methods (Analytical and Bioanalytical) for precise BHC estimation in varied matrices, addressing crucial needs in pharmaceutical and biomedical research.Utilizing a luminescence spectrometer (Perkin Elmer, UK), BHC was detected with 1M Methanolic H_2SO_4, at excitation and emission wavelength of 350 nm, and 530 nm respectively. BHC extraction from spiked human plasma employed a protein precipitation method with acetonitrile. Validation studies, following ICH Q2 (R1) guidelines, demonstrated the efficacy of both methods. Linear responses were observed in the 200-800 ng/mL BHC concentration range, with precision (% RSD <5%) meeting acceptable limits. Recovery studies (90%-110%) affirmed reliability and reproducibility. The developed spectrofluorophotometric methods for BHC estimation offer simplicity, linearity, sensitivity, precision, and accuracy. Their robustness, confirmed through validation and comparison with existing methods, positions them as valuable tools for BHC quantification in pharmaceutical and biomedical research. These methods present a promising advancement in analytical approaches for researchers in the field.

KEYWORDS: Berberine hydrochloride; isoquinoline alkaloid; analytical and bioanalytical method; spectrofluorophotometry; human plasma.

1. INTRODUCTION

Berberine hydrochloride (BHC), a yellow fluorescent isoquinoline alkaloid, has attracted significant research interest due to its diverse pharmacological properties and therapeutic potential, including cardiovascular benefits, anti-inflammatory, antioxidant, anticancer, and antibacterial effects [1–7]. Effective against bacteria, viruses, fungi, helminths, protozoans, and chlamydia, it enhances its utility in treating infections [8–10]. Derived from medicinal plants such as *Berberis species*, *Phellodendron amurense*, and *Coptis chinensis*, significant in traditional Chinese medicine and Ayurveda, for addresses conditions like diabetes, infectious diseases, and gastrointestinal issues [11]. Its numerous benefits have led to widespread use in nutritional supplements [12].

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This compound possesses a distinctive quaternary ammonium group-containing structure [Figure 1] and lipophilic nature that enhance cellular permeability and absorption [13]. Its ability to cross the blood-brain barrier (BBB) allows for central nervous system (CNS) protection when administered systemically [14,15]. Additionally, it improves glucose and lipid metabolism by increasing insulin sensitivity, promoting glucose uptake, and improving lipid profiles, thus offering therapeutic potential for metabolic disorders such as diabetes and dyslipidemia [16–18]. However, exceeding recommended doses can cause adverse digestive effects [19]. In cancer research, it shows promise by inhibiting cell growth, inducing apoptosis, and suppressing metastasis through pathways such as NF-κB, PI3K/Akt, AMP-activated protein kinase (AMPK), and MAPK [20–23].

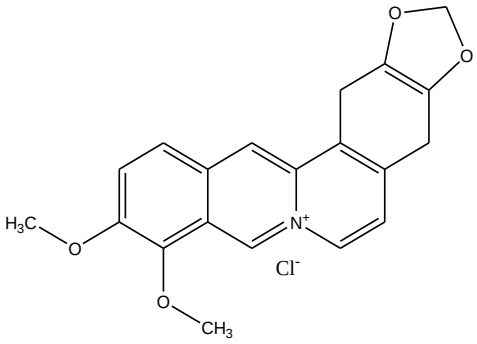


Figure 1. Chemical structure of BHC

Metabolism of BHC occurs primarily in the liver by cytochrome P450 enzymes (1A2, 2D6, and 3A4) and UDP glucuronosyltransferases. Phase I metabolism involves demethylation, while Phase II involves glucuronidation, yielding metabolites like demethylene-berberine, berberrubine, thalifendine, jatorrhizine, and their glucuronidated derivatives [24,25].

The rising demand necessitates precise analytical techniques to detect BHC in bulk samples, marketed formulations, and biological fluids. Methods such as UV-Vis spectroscopy [26,27], High-Performance Thin-Layer Chromatography (HP-TLC) [28,29], High-Performance Liquid Chromatography (HPLC) [30–32], and Liquid Chromatography/Mass Spectrometry (LC/MS) [33,34] face challenges like low sensitivity, time consumption, and high costs [35]. Spectrofluorophotometry, with its high sensitivity, simplicity, and selectivity, has emerged as a promising alternative [36]. By measuring fluorescence emitted upon light stimulation at specific wavelengths, this technique enables accurate measurement even in complex matrices [37,38].

This study aims to develop and validate a spectrofluorophotometry technique for assessing BHC in bulk material, commercial formulations, and spiked human plasma, leveraging its fluorescent properties. The developmental approach optimizes experimental parameters, including excitation and emission wavelengths, while subsequent validation encompasses assessments of linearity, sensitivity, reliability, precision, and accuracy. This research provides a rapid and sensitive analytical tool for determining this compound, applicable in quality control laboratories, pharmacokinetic studies, and bioequivalence assessments. By validating the method across various sample matrices, we ensure its applicability throughout the pharmaceutical manufacturing process, from raw material analysis to formulation evaluation [39,40].

2. RESULTS AND DISCUSSION

2.1. Method Optimization

The solubility and fluorescence intensity of BHC was maximum observed in 1M methanolic H_2SO_4. The emission fluorescence spectra and calibration curve of BHC in pure form and in spiked human plasma showed linearity in the range of 200-800 ng mL^{-1} at 530 nm.

2.2. Extraction Efficiency of Drug from human plasma

The extraction efficiency was found to be in the range of 89.70% to 95.75% which indicates that the optimized extraction procedure was efficient for the extraction of drug from human plasma. Hence, optimized protein precipitating extraction method was utilized for further procedures.

2.3. Method Validation

All validation parameters for method A and method B were used to validate the purposed approach in accordance with the ICH Q2 (R1) standard [41]. A summary of the analytical method validation parameter determination can be found in **Table 1**.

Table 1. Summary of analytical method validation

Parameter	Method A	Method B
Linear and range		
Range (ng mL^{-1})	200 - 800	200 - 800
Regression equation	y = 0.1785x + 122.26	y = 0.1693x + 120.04
Correlation coefficient	0.9976	0.9937
Slope	0.1785	0.1693
Intercept	122.26	120.04
LoD (ng mL^{-1})	41.96	64.34
LoQ (ng mL^{-1})	127.18	194.99
Accuracy	97.60% to 98.13%	92.47% to 93.69%
Precision (%RSD)		
Intraday	0.06 - 0.13 %	0.11 - 0.54 %
Interday	1.22 - 1.99 %	0.23 - 1.01 %
Assay (%)	98.74 %	91.67

2.3.1. Linearity and Range

The overlay spectra was taken at 570 nm for series of dilutions (200-800 ng mL^{-1}) for both the methods [Figure 2(a) and 2(b)] and a calibration curve was constructed for both methods shown in [Figure 3(a) and 3(b)] by plotting the BHC concentration (ng mL^{-1}) on the x-axis against the fluorescence intensity on the y-axis. The curve demonstrated a linear relationship within the concentration range of 200 to 800 ng mL^{-1}. To evaluate the reliability of the method, the study was replicated six times, resulting in a %RSD of less than 2%, indicating its suitability for analyzing BHC in plasma/serum and solution matrices.

2.3.2. LoD and LoQ

To calculate Limit of Detection (LoD) and Limit of Quantification (LoQ), the calibration curve's correlation coefficient, slope, and intercept were employed with the following equations.

$LoD = 3.3*\sigma / S$

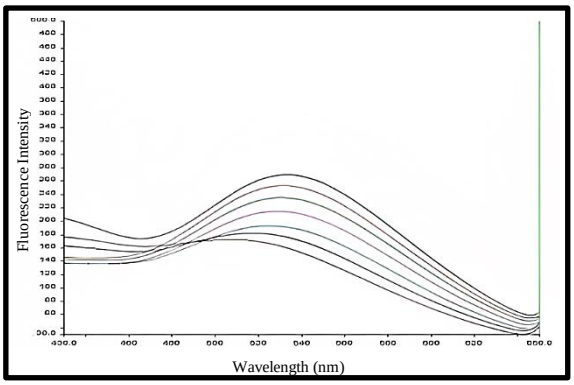
$LoQ = 10*\sigma / S$

Where, σ = SD of the y-intercept and S = slope

The LoD and LoQ values achieved for methods A and B were notably low, with 41.96 ng mL-1 and 127.18 ng mL-1 for Method A, and 64.34 ng mL-1 and 194.99 ng mL-1 for Method B, respectively. As reflected in the results, these lower values for LoD and LoQ indicate the heightened sensitivity displayed by the method.

2.3.3. Accuracy

To ensure the accuracy of the proposed procedures, samples containing spiked standards at three levels (80%, 100%, and 120%) were prepared using marketed formulation and human plasma for method A and method B, respectively. These spiking levels were repeated three times to validate the reliability of the methods. The obtained results indicated a close agreement between the measured values and the actual values, as demonstrated by the percentage recoveries achieved. The % recovery for Method A varied between 97.60% and 98.13%, while Method B fluctuated within 92.47% to 93.69% [Table 2]. These results provide additional evidence reinforcing the accuracy and exactness of both Method A and Method B.



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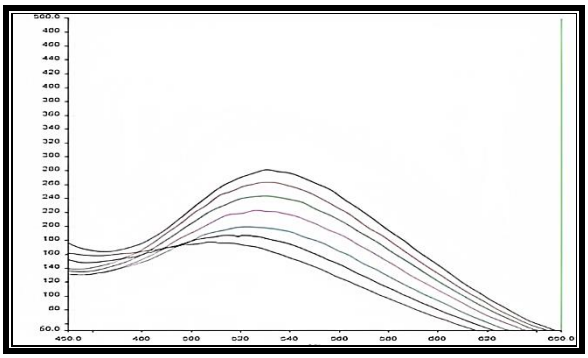
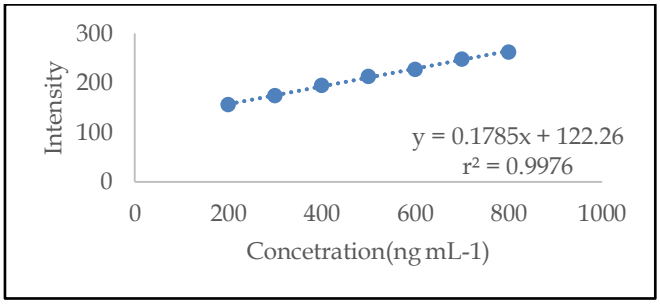


(b)

Figure 2. (a) Overlay spectra of BHC at 570nm (200-800 ng mL^{-1}) and (b) Overlay spectra of BHC in spiked human plasma at 570nm (200-800 ng mL^{-1})



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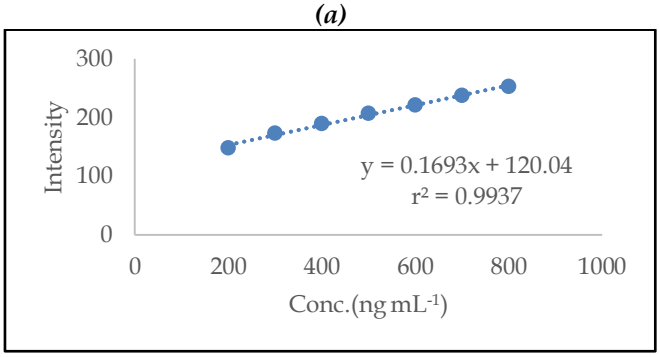


(b)

Figure 3. (a) Calibration Curve for BHC at 570nm (200 – 800 ng mL^{-1}) and (b) Calibration Curve for BHC in spiked human plasma at 570nm (200 – 800 ng mL^{-1})

2.3.4. *Precision*

To assess the precision of the suggested procedures, both interday and intraday precisions were evaluated. This was achieved by conducting three repetitions at three concentrations spanning the range of 200 to 800 ng mL^{-1}. The proposed methods A and B demonstrate significant reproducibility and reliability, as evidenced by the impressively low %RSD values. Method A exhibits a range of 0.06 to 0.13% for intraday precision, while Method B falls within 0.11 to 0.54%. Interday precision similarly shows low variability with Method A ranging from 1.22 to 1.99% and Method B from 0.23 to 1.01% [Table 2]. These consistently low %RSD values underscore the high level of reproducibility and dependability inherent in these methods

Table 2. Evaluation of accuracy and precision for determination of BHC using proposed methods

Parameter		Method A			Method B		
Accuracy							
Level	**Amount taken (ng mL^{-1})**	**Amount obtained (ng mL^{-1})**	**% Recovery for method A**		**Amount obtained (ng mL^{-1})**	**% Recovery for method B**	
80%	540	520.22	97.60		499.33	92.47	
100%	600	570.08	98.13		562.14	93.69	
120%	660	631.59	97.72		611.42	92.64	
Precision							
		200 ng mL^{-1}	**500 ng mL^{-1}**	**800 ng mL^{-1}**	**200 ng mL^{-1}**	**500 ng mL^{-1}**	**800 ng mL^{-1}**
Intraday	1	157.25	216.45	259.10	148.25	207.45	252.19
	2	156.86	216.34	259.72	149.86	208.34	251.72
	3	156.91	216.61	259.66	148.91	207.61	251.66
	Mean	157.00	216.46	259.49	149.00	207.80	251.85
	SD	0.21	0.13	0.34	0.80	0.47	0.29
	%RSD	0.13	0.06	0.13	0.54	0.22	0.11
Interday	1	157.25	216.45	259.10	148.25	207.45	252.19
	2	157.26	217.81	260.68	151.26	208.81	252.68
	3	164.79	222.90	266.56	152.79	207.90	254.56
	Mean	159.48	218.89	262.02	150.69	208.19	253.03
	SD	3.18	2.68	3.34	1.52	0.48	1.17
	%RSD	1.99	1.22	1.27	1.01	0.23	0.46

2.3.5. *Assay of Marketed Formulation (capsules)*

The established methodologies were employed to measure BHC in commercial pharmaceutical formulations quantitatively. Table 3 presents the assay percentage results for Methods A and B, showcasing respective values of 98.74% and 91.67% respectively.

Table 3. Evaluation of marketed formulation of BHC using proposed methods

Conc. (ng/mL)	Method A			Method B		
	Mean Intensity	**%Assay**	**%RSD**	**Mean Intensity**	**%Assay**	**%RSD**
300	174.60	98.74	0.43	166.93	91.67	0.45

2.4. Comparison of the Developed Method with already published methods

In this groundbreaking research article, we unveil a novel spectrofluorophotometric method meticulously developed for the precise quantification of berberine hydrochloride across bulk samples, marketed formulations, and spiked human plasma. During the course of our research, we drew valuable insights from a comprehensive article that compiled a thorough review of analytical and bioanalytical methods, enriching our understanding and contributing to the innovative development of the spectrofluorophotometric method for berberine hydrochloride quantification [13]. Our method not only surpasses conventional techniques but also underscores its unparalleled sensitivity and efficacy, particularly in the detection of minute concentrations of berberine hydrochloride. This advancement in pharmaceutical and biomedical research is pivotal, highlighting the method's exceptional capability to discern berberine hydrochloride at lower levels. Importantly, our innovative approach champions sustainability by requiring

fewer solvents, aligning with eco-friendly and cost-effective analytical practices. Rigorous validation substantiates the unwavering reliability and accuracy of our method, positioning it as the preferred choice for routine berberine hydrochloride analysis across diverse matrices. This research not only validates the efficacy of our spectrofluorophotometric method but also charts a transformative course for future analytical advancements in the quantification of berberine hydrochloride, setting a new standard in conventional methodologies.

3. CONCLUSION

The research has successfully developed and validated a spectrofluorophotometric method for the quantification of Berberine hydrochloride (BHC) in different matrices including bulk samples, commercial formulations, and spiked human plasma. The method utilized the inherent luminescent properties of BHC and optimized experimental parameters such as excitation and emission wavelengths. The validation parameters demonstrated a high level of linearity, sensitivity, precision, and accuracy, affirming the method's reliability. Both analytical techniques demonstrated remarkable linearity within the 200 to 800 ng mL^{-1} concentration range. The proposed methods exhibited exceptional sensitivity, as indicated by the remarkably low LoD and LoQ values, attesting to the method's ability to detect and quantify even minuscule amounts of BH and precision, as shown by the impressively low %RSD values. The method's accuracy was further confirmed by high percentage recovery values. Upon application to commercial pharmaceutical formulations, the methods delivered satisfactory results, underscoring their applicability in practical scenarios. In essence, by comparing the developed method with published conventional method, it represents a swift, sensitive, and reliable analytical tool for BHC determination, which can be effectively used in various stages of pharmaceutical production and quality control procedures.

4. MATERIALS AND METHODS

4.1. Apparatus

(a) Spectrofluorimetric determination - Luminescence spectrometer (model LS 55, Perkin Elmer, UK) employed with a 1 cm quartz cuvette, outfitted with a Xenon 150W lamp, a 1 cm quartz cell, and 15 cm monochromators (excitation and emission). The monochromatic slit width for excitation and emission were set at 5nm. The FL Winlab program was utilised to do the measurements.
(b) Digital weighing balance (Mettler Toledo, Switzerland)
(c) Bath Sonicator (Vijay Scientific)
(d) Hot air oven (Vijay Scientific)
(e) Centrifuge (Remi)
(f) Vortex mixer (Remi)

4.2. Chemical and Reagent

(a) BHC - purchased from Yucca Enterprises (Mumbai).
(b) Analytical solvents - such as methanol, hydrochloric acid, sodium hydroxide and sulphuric acid of HPLC grade were procured from LOBA Chemie Pvt. Ltd., Mumbai, India.
(c) Milli-Q water
(d) Berberine HCl capsule (Nutrija Lifesciences) - label clime of 500 mg BHC/capsule purchased from local market.

4.3. Selection of Diluent

Several diluents were examined to increase relative fluorescence intensity, including water, Methanol, 0.1-1 M H_2SO_4 (in water), 0.1-1 M HCl (in water), 0.1-1 M NaOH (in water), 0.1-1 M methanolic HCl, 0.1-1 M methanolic NaOH, and 0.1-1 M methanolic H_2SO_4. Amongst all, methanolic H_2SO_4 had the highest relative fluorescence intensity of any diluent. Hence, methanolic H_2SO_4 was selected as a diluent for throughout the study.

4.4. Selection of Diluent Concentration

BHC (10mg each) was dissolved individually in 0.25M, 0.5M, 1M and 1.5M methanolic H_2SO_4. In comparison to other concentrations, BHC gave maximum intensity at 1 M Methanolic H_2SO_4. As a result, 1M Methanolic H_2SO_4 was chosen as a diluent for future investigation [**Figure 4**].

4.5. Standard Solution Preparation

BHC (10mg) was accurately weight and transfer into 10mL of volumetric flask and diluted with 1M methanolic H_2SO_4 solution to get 1 mg mL^{-1} of BHC. From that 1ml of aliquot was transferred into 10 ml volumetric flask and the marks was made up with 1 M Methanolic H_2SO_4 solution to get the standard stock solution of BHC(100 µg mL^{-1}). From the resulting solution, 1 ml was transferred into 10 ml volumetric flask and dilute up to the mark with 1 M Methanolic H_2SO_4 solution to get working standard solutions (WSS) of 10 µg mL^{-1} (10,000 ng mL^{-1}).

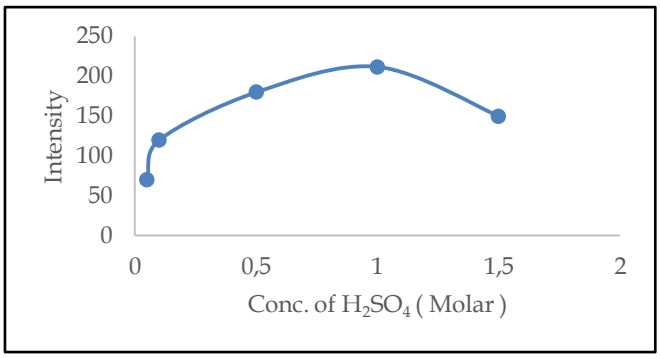


Figure 4. Effect of H_2SO_4 concentration on fluorescence intensity

4.6. Detection of Excitation and Emission Wavelength

10 µg mL^{-1} solution of BER was prepared using 1M methanolic H_2SO_4 and scanned in UV Spectrophotometer and spectrum was recorded, which shows absorbance at 350 nm [**Figure 5(a)**]. The resulting solution again scanned over the range of 370-800 nm against excitation wavelength 350 nm in Spectrofluorophotometer and spectrum was recorded, which shows emission maxima at 530 nm [**Figure 5(b)**]. To counter check the process, the solution was again scanned over the range of 200- 370 nm against the emission wavelength selected as a 530 nm in Spectrofluorophotometer. The maximum intensity of excitation wavelength was showed at 350 nm. Hence, $\lambda_{exi}/\lambda_{emi}$ at 350/530 was used further throughout the study.

4.7. Method Validation

Both the methods; Analytical (Method A) and Bioanalytical (Method B) were validated as per ICH Q2 (R1) guideline [41].

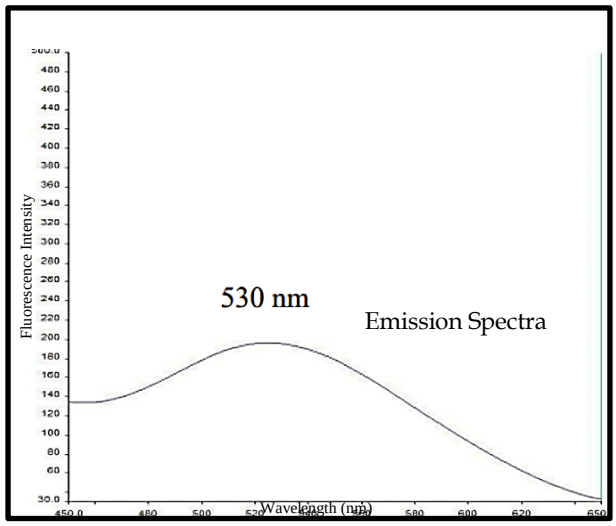
4.8. Calibration curve of BHC (Method A)

Different volumes ranging from 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 and 0.8 ml of working stock solution were transferred in to a series of 10 mL volumetric flasks and diluted upto the mark with 1M methanolic H_2SO_4 solution to get a series of standard solutions (200-800 ng/mL) for calibration curve.

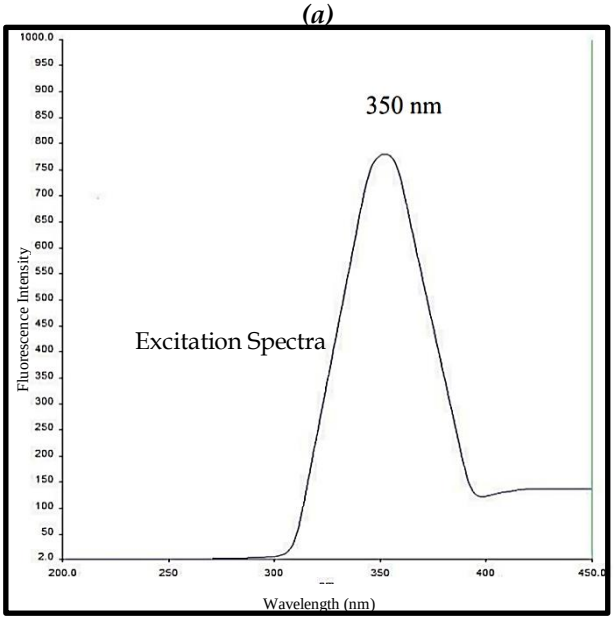
4.9. Calibration curve of BHC in spiked human plasma (Method B)

BHC has a protein binding capability of higher than 90%. As a result, the protein precipitating extraction technique was employed to extract BHC from spiked human plasma. As a protein precipitating agent, acetonitrile (ACN) was utilised. ACN volumes of 1, 1.5, 2, and 2.5 mL were employed in the trials. The samples containing 2 mL and 2.5 mL ACN produced the best results and there was no change in the results of samples observed with the use of 2 and 2.5 mL ACN. As a result, 2 mL ACN quantity was fixed to use as a precipitating agent in all further processes.

Suitable aliquots of working stock solution (ranging from 0.21, 0.31, 0.42, 0.52, 0.63, 0.73, and 0.84 mL of working stock solution were transferred in to a series of 10 mL volumetric flasks using 1mL micro pipette (Thermo Fisher Finnpipetter F3) and dilute upto the mark using 1M methanolic H_2SO_4 to give final concentration of 4200-16800 ng mL^{-1} for spiking solution. 0.2 mL of each spiking solutions of above mentioned concentration was added to 2 mL of blank plasma in the tarson tube. The solution was vortex for 5 min using vortex mixer then 2 mL of ACN was added. The mixture was shaken again on a vortex mixer for 2 min, and followed by centrifuging it at 3000 rpm for 45 min. At the end supernatant solution was collected of final concentration range of 200-800 ng mL^{-1}.



(a)



(b)

Figure 5. (a) Emission Spectra at 350 nm Excitation Wavelength and (b) Excitation Spectra at 530 nm Emission Wavelength

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Conflict of interest statement: The authors declare no conflict of interest.

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