Preconcentration of rifampicin prior to its efficient spectroscopic determination in the wastewater samples based on non-ionic surfactant

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Abstract

Every year, tuberculosis affects the lungs of millions of people and rifampicin is the commonly used medicine for its treatment due to its antibiotic nature. The frequent use of rifampicin may lead to its increased concentration in the water resources. This research work is focused on the cloud point extraction (CPE) procedure for the preconcentration of rifampicin prior to its determination in water. The UV/*Vis* spectrophotometric method was adapted for the measurement of rifampicin content after the phase separation. Triton-X 100 was used as the non-ionic surfactant which contains hydrophilic polyethylene chain feasible for the extraction of analyte. Various analytical parameters that can affect the extraction efficacy were optimized to get linearity of the proposed method in the concentration range of 3.54-81.41 mgL^{-1}. The Limit of detection and quantification were 1.261 and 4.212 mgL^{-1}, respectively. The Preconcentration factor was 40 with relative standard deviation (%RSD) of 2.504%. The standard addition methodology was adopted for the validation of this procedure and effectively applied for the determination of rifampicin in real wastewater samples.

Key words: Antibiotic, cloud point extraction, preconcentration, rifampicin, wastewater

1. Introduction

Tuberculosis (TB) is a lung infection disease and one of the major causes of death until the end of twentieth century in many countries [1]. TB has been realized as a great threat for human being all over the world like the HIV and malaria, therefore, in 1993 World Health Organization declared TB as the great global threat. In 2005, 12 million TB cases were observed and 1.5 million deaths occurred due to TB [2]. For the treatment of TB patients, different antibiotics such as rifampicin, isoniazid etc. have been widely used [3].

Rifampicin (RIF) introduced in 1972 as an antitubercular agent [4], belongs to special macrocyclic group of antibiotics effective against *Mycobacterium* tuberculosis [5]. Beside this RIF plays vital role during sporadic stage to kill semi dormant Tuberclue *bacilli* [6, 7]. Due to the presence of macrocyclic ring in RIF, which is considered as key structural unit, interacts with the microbial DNA-dependent RNA polymerase and inhibits the introduction of RNA synthesis [8, 9]. RIF can be toxic for biological system to produce several side effects like, allergic rashes, nausea, hepatotoxicity, appetite loss, immunological disturbances [10], oxidative conjunctivitis [11], fatigue, headache [12] and organic brain syndrome [13].

Anti-TB drugs in different samples have been determined with a number of instrumental techniques like, high performance liquid chromatography (HPLC) [14], infrared spectroscopy [15], liquid chromatography and gas chromatography coupled with the mass spectrometry [14]. Liquid chromatography-tandem mass spectrometry for rifampicin determination has been widely used with low detection limit in environmental samples [16]. The traditional extraction techniques used for the

extraction and removal of drugs in water samples have some limitations such as time consumption and use of excessive toxic organic solvents (especially, in case of liquid-liquid extraction and solid phase extraction) [17]. Recently, the focus has been shifted towards more economic and efficient techniques requiring least utilization of solvents and shorter analysis time as well as maximum preconcentration factor [18].

Increase in the development of surfactant-based extraction methods in sample preparation is observed during the last few decades where the most widely explored surfactant-based preconcentration process is the cloud point extraction (CPE). CPE rely on the phase behavior of nonionic and zwitter ionic surfactants in aqueous solutions which exhibit phase separation upon temperature change or addition of salt [19]. CPE is eco-friendly process because this technique follows the principles of green chemistry, by utilizing non-volatile surfactants with least toxicity than those organic solvents [20]. Moreover, CPE has been widely utilized in separation science for extraction, purification and preconcentration of different analytes prior to their analysis by UV/*Vis* spectrophotometry. The UV/*Vis* spectrophotometry is well-known for its simplicity, reliability and low cost operation. These features make UV/*Vis* spectrophotometry a good choice against sophisticated detection techniques [20]. Therefore, the aim of this work was to develop a quick, selective and simple CPE method for the preconcentration of RIF in real wastewater samples.

2. Experimental

2.1. Reagents and Chemicals

Water for experimental work was purified on water distillation apparatus IM-50 (IRMECO GmbH & Co. KG, Schwarzenbek-Germany). The fresh working standard

solutions for RIF were prepared on daily basis through stepwise dilution of the stock standard solutions purchased from Merck, Darmstadt, Germany. HPLC grade ethanol was purchased from BDH Laboratory Supplies (England) while sodium chloride was obtained from (Scharlab S.L, Barcelona-Spain). Nonionic surfactant Triton X-100 was obtained from Merck (Darmstadt, Germany) and was used without further purification. A 2.5% (v/v) nonionic surfactant solution was prepared by dissolving 2.5 mL of Triton X-100 (Merck, Darmstadt, Germany) in 100 mL distilled water.

2.2. Instrumentation

Absorbance measurements were performed with double beam UV/*Vis* spectrophotometer (PG Instruments Limited, BMS, UK) equipped with deuterium and tungsten lamp. For the pH adjustments, pH meter (Adwa Instruments Kft. Szeged-Hungary) was used. Centrifuge machine (Eppendorf AG, 22331 Hamburg-Germany) was employed for the centrifugation of samples. The constant temperature water bath (Thermostat, DAHAN Scientific, North America, WB-22) was used to maintain temperature required for phase separation.

2.3. Samples

Wastewater samples were collected from different places (suburbs and inside the Abbottabad city). The samples were then filtered using a vacuum filter funnel (porosity 25–50 μm, Aldrich) to remove any suspended particulate matter.

2.4. CPE Procedure

For CPE preconcentration, the aliquots (20 mL) standard solutions containing 15 mgL^{-1} RIF (pH=9) containing 1.0 mL of Triton X-100 (2.5% v/v) were transferred

into polypropylene tubes of 50 mL capacity and then heated at constant temperature of 65°C (5 min). Later to achieve phase separation, the mixture was centrifuged at 3500 rpm for 5 min. Then samples were cooled inside a refrigerator at 0°C (5 min) to increase the viscosity of Triton X-100, the upper phase was carefully removed by pipette. The viscosity of coacervated phase was lowered by adding 2 mL ethanol. This helped us to have the required solution volume essential for the UV/*Vis* spectrophotometry. Standard solution of RIF was scanned in visible range of 300-550 nm to find wavelength (λ_{max}) for maximum absorbance Figure **S1**. All the measurements were carried out at the constant wavelength (λ_{max}) of 338 nm for subsequent analysis.

3. Results and Discussions

3.1. Optimization of experimental parameters

3.1.1. Effect of pH

The influence of pH value on speciation of organic compounds in aqueous sample will lead to affect the extraction proficiency. The optimum pH value for RIF recovery were investigated in the range of 3 to 11, while the other parameters were kept constant. The results showed that appreciable improvement of RIF recovery was observed in the alkaline range Figure 1. Then it was decreased at pH greater than 9. The extraction efficiency of RIF decreases in acidic solutions due to the protonation of nitrogen in structure of RIF Figure **S2**. Therefore, pH 9 is the optimum value selected for the subsequent experiments.

3.1.2. Effect of temperature and incubation time

Cloudpoint determines the temperature of the phase inversion (clarity/cloudiness). Temperature above the 'cloud point' is called cloud point temperature. Therefore, we observed maximum solubilizing ability of analyte for the Triton X-100 at 65°C due to its 67°C cloud point temperature [21]. However, it is desirable to employ the lowest possible equilibration temperature and shortest incubation time, to complete the reaction and efficient separation of phases. For better phase separation the temperature parameter was investigated (20–75°C). The Figure 2, indicates that the highest recovery was obtained at 65°C, thus this temperature was chosen for subsequent experiment in order to achieve quantitative recovery.

3.1.3. Effect of ionic strength

The addition of NaCl into the sample solution can enhance the phase separation phenomenon of aqueous phase and surfactant rich phase. The aggregation and micelle size is enlarged as the concentration of NaCl increased but critical micellar concentration (CMC) remains constant [22]. The effect of NaCl concentration on preconcentration factor and overall recovery was examined in the range of 0.1-0.6 $mol.dm^{-3}$ Figure 3. It appeared that, extraction efficiency of RIF was quantifiable while using 0.4 $mol.dm^{-3}$. In this way, 0.4 $mol.dm^{-3}$ was utilized in the subsequent experiments.

3.1.4. Effect of Triton X-100 concentration

For an effective CPE, the extraction efficiency can be enhanced by minimizing the surfactant phase volume, thereby ensuring the highest preconcentration factor. The usefulness of Triton X-100 concentration in preconcentration efficacy was examined

in the range of 0.5-5% (v/v). The maximum absorbance was observed at 2.5% (v/v). Figure 4 highlights that quantitative recovery was reduced by increasing the surfactant concentration due to the loss of UV/*Vis* spectrophotometer signal. Thus, 2.5% (v/v) concentration was chosen for the subsequent experiments.

3.1.5. Effect of sample volume

The sample volume plays vital role in the preconcentration during the analysis of real samples for obtaining good yield [23]. The effect of sample volume on the extraction efficiency was examined in the range of 5-50 mL. The results Figure 5, showed the extraction efficiency of RIF was almost same up to 20 mL, but reduced with higher sample volumes. The slight decrease in the extraction efficiency is due to insufficient interaction between the analyte and extractant phase. On the basis of these results, 20 mL of sample volume was chosen for subsequent experiments.

3.1.6. Effect of centrifugation rate and centrifugation time

Centrifugation rate and time affect the efficiency of CPE as these factors play vital role in phase separation of surfactant from aqueous phase [24]. It is aimed to preconcentrate RIF; centrifugation rate was studied in the range of 1000–5000 rpm. The results in Figure 6 indicates that maximum recovery occur at 3500 rpm and no further improvement was observed for prolonged period. The influence of the centrifugation time (1 to 10 min) on CPE of RIF was also examined. The results revealed that significant recovery was observed at 5 min which was selected for further experiments Figure 7.

3.2. Interference study

The presence of coexisting species in the real samples and their interference effects are well known in the instrument based analysis [25]. In present study, the tolerable error is defined as the effect of interferences on the extraction of RIF were investigated. The maximum number of coexisting species that generate an error not beyond 5% during investigation of RIF are termed as tolerable limit. There can be different interferences, such as isoniazid, pyrazinamide and ethambutol attributed to the analytical signal of RIF. Stock solutions of 20 $mg.mL^{-1}$ of RIF were prepared in the presence of isoniazid, pyrazinamide and ethambutol was subjected to the CPE procedure and finally determined by UV–*Vis* spectrophotometry. Resulting data indicates in Table 1, that no considerable effect of the tested drugs is observed on RIF signals.

3.3. Validation of proposed CPE through standard addition method

Standard addition method was used to validate the applicability of proposed method. Real wastewater samples were analyzed after the addition of known quantity of drugs for the determination of RIF through proposed CPE method followed by the UV/*Vis* spectrophotometer. The recoveries of RIF Table 2 were quantifiable for trace analysis and no noticeable matrix was observed.

3.4. Analytical figures of merit

It is very important to examine the accuracy and precision of the proposed method, therefore, this method was utilized for the preconcentration followed by determination of RIF in the wastewater samples. As illustrated in Table 3, the recoveries of RIF were

found reasonable under the optimized experimental conditions. The linear regression, calibration equation, correlation coefficient, limit of detection (LOD), limit of quantification (LOQ), and preconcentration factor are summarized in Table 3. The linear concentration was in the range of 4.12-81.41 mgL^{-1}.

The extraction recovery (*ER*) was calculated according to the following equation:

$$ER = \frac{m_{surfactant}}{m_{aq}} = \frac{C_{surfactant} \times V_{surfactant}}{C_{aq} \times V_{aq}} \times 100$$

The $m_{surfactant}$ is the concentration of analyte in the final surfactant phase while m_{aq} is the initial concentration of analyte in the sample solution, $C_{surfactant}$ and C_{aq} are the analyte concentration in surfactant phase and in the aqueous phase, respectively. $V_{surfactant}$ and V_{aq} are the concerned volumes of the phases. The LOD and LOQ were calculated by the 3 *s*/*m* and 10 *s*/*m*, respectively, where ***s*** represents standard deviation from 10 blank measurements and *m* is the slope of the calibration curve. The LOD and LOQ obtained were 1.261 and 4.212 mgL^{-1}, respectively. The preconcentration factor (PF) was calculated as the ratio between the initial volume (sample) and the final volume.

$PF = V_{(initial)}/V_{(final)}$

Excellent analytical figures of merit were obtained with proposed procedure while utilizing more simpler instrument (i.e. UV/*Vis* spectrophotometer) and the results can be compared with some literature reported techniques Table 4 [26-31].

4. Conclusions

This research demonstrated the optimized, efficient cloud point extraction method for the selective extraction, preconcentration and detection of RIF from the wastewater samples. The proposed method allowed the determination of minute quantity of RIF in real samples by UV/*Vis* spectrophotometer which is available in most of the laboratories. Cloud point extraction *via* Triton X-100 offered sensitive, selective, rapid, reproducible, and inexpensive alternative to other separation and preconcentration techniques. The proposed procedure resulted in good precision, accuracy and better limit of detection. As compared to other separation procedures, this technique is highly eco-friendly. It was observed that the addition of salt is effective in phase separation to prevent the possible loss of analyte during heating.

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Table 1. Effect of interfering drugs on the recovery of RIF (N=7).

S. No	Interferences	Tolerance Limit (mgL^{-1})	% Recovery of RIF ($150\ mgL^{-1}$)
1	Pyrazinamide	400	99.1 ± 1.25
2	Ethambutol	275	
3	Isoniazid	75	

Table 2. Standard addition methodology for the validation of CPE (N=7).

Sample	Added amount (mg)	Concentration (mgL^{-1})	Found (mgL^{-1})	% Recovery
Wastewater	0.0	0.0	BDL	---
	0.2	10	9.89 ± 0.61	98.9 ± 6.11
	0.4	20	19.84 ± 0.73	99.2 ± 7.30

BDL: Below detection limit

Table 3. Analytical figures of merit for proposed CPE methodology (N=7).

Linear range (mgL^{-1})	4.21-81.41
Calibration equation (mgL^{-1})	A = 0.0294C + 0.0199
Correlation coefficient (R^2)	0.998
Limit of detection (mgL^{-1})	1.261
Limit of quantification (mgL^{-1})	4.212
% Relative standard deviation	2.504
Preconcentration factor	40

Table 4. Comparison of the proposed CPE methodology with other reported technique for the determination of RIF.

Sample Preparation Technique	Detection Technique	Sample	Detection limit	Linear Range	% R.S.D	% Recoveries	Reference
Liquid–liquid extraction	HPLC–UV	Human plasma	-	1–50 mgL^{-1}	15	83	26
		Liver sample	-	0.6–40 $\mu g\ g^{-1}$	15	95	
Glutathione-capped CdTe/ZnS QDs	Fluorescence	Aqueous solution	0.25 $mg\ mL^{-1}$	0.83– 56 $mg\ mL^{-1}$	-	98.6 - 103.2	27
Liquid extraction	Reversed phase HPLC-UV	Human plasma	-	2–20 mgL^{-1}	5 – 23	91.73	28
		Human urine	-	20–200 mgL^{-1}	5 – 23	96.24	
None	HPLC–UV	Pharmaceutical	0.2 mgL^{-1}	1–40 mgL^{-1}	≤ 2.5	99.7 - 100.5	29
Strata-X-CW extraction cartridge	HPLC–UV	Human plasma	0.15 mgL^{-1}	0.5–20 mgL^{-1}	≤ 8.0	84.5	30
		Human blood spot	1.5 mgL^{-1}			65.0	
Glassy carbon electrode/nanostructured nickel hexacyanoferrate	Voltammetry	Human urine	2.6 $\mu mol\ L^{-1}$	5.0×10^{-6} - 5.0×10^{-4} $mol\ L^{-1}$	4.2	97.0 - 102	31
CPE	UV/Vis spectrophotometry	Wastewater	1.261 mgL^{-1} (1.53 $\mu mol\ L^{-1}$)	4.21-81.41 mgL^{-1}	2.504	99.2	Present

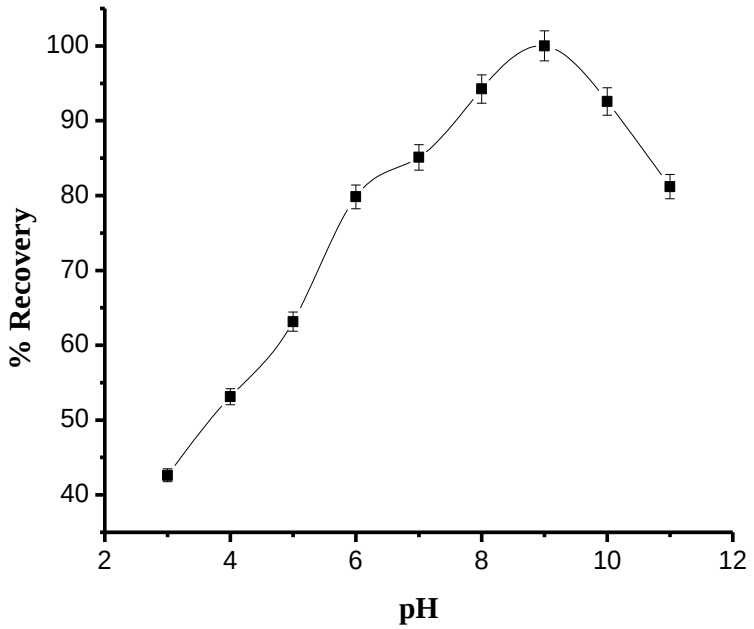

Figure 1. Effect of pH on RIF %recovery (N=7).

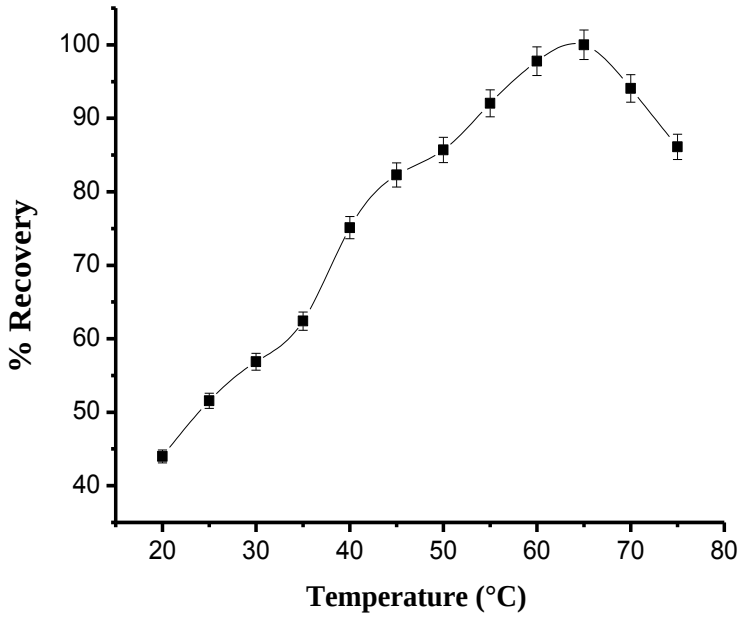

Figure 2. Effect of temperature (°C) on RIF %recovery (N=7).

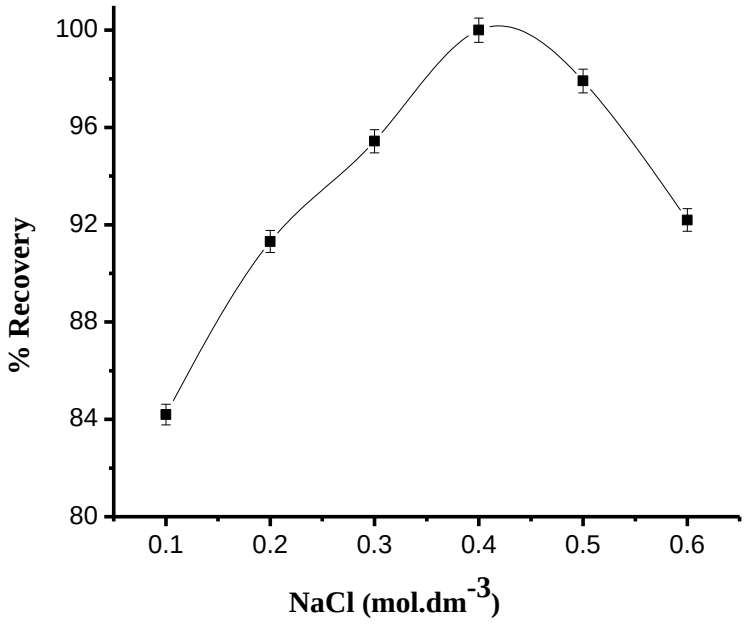

Figure 3. Effect of ionic strength on RIF %recovery (N = 7).

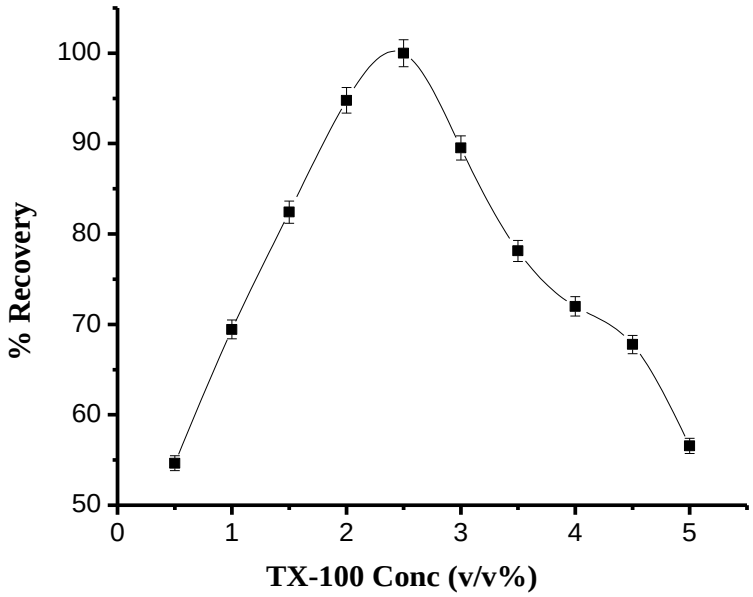

Figure 4. Effect of TX-100 concentration (v/v%) on RIF %recovery (N=7).

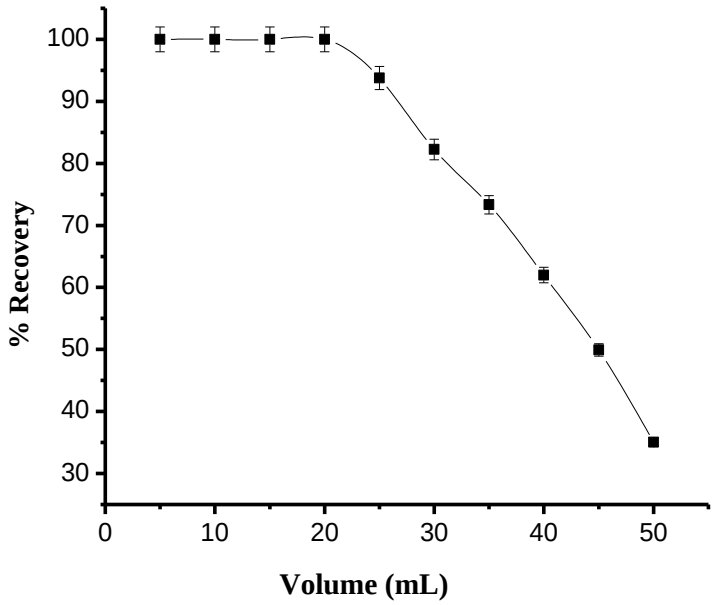

Figure 5. Effect of sample volume on RIF %recovery (N=7).

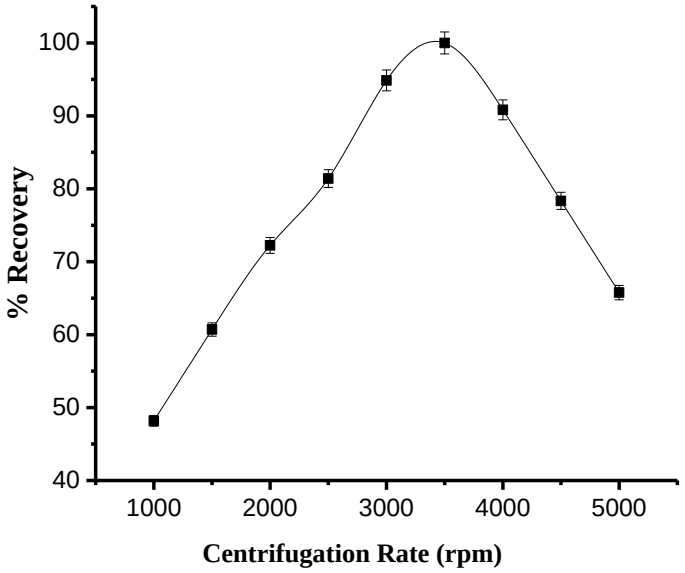

Figure 6. Effect of centrifugation rate on RIF %recovery (N=7).

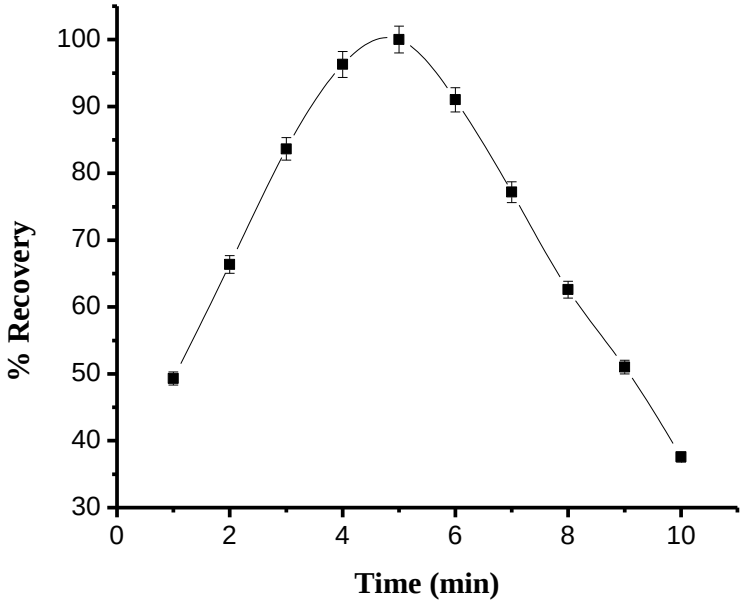

Figure 7. Effect of centrifugation time on RIF %recovery (N=7).

Supporting Material:

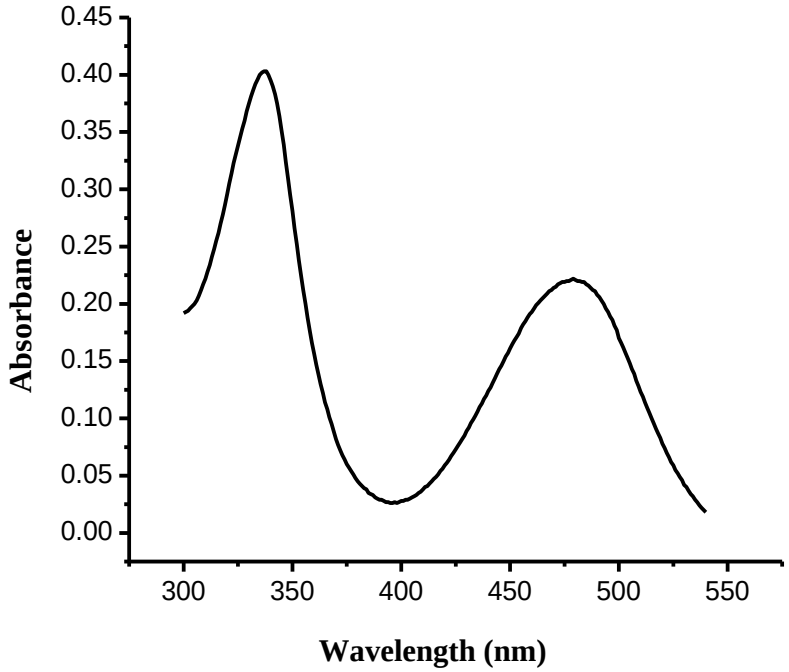

Figure S1: Visible absorption spectra for RIF.

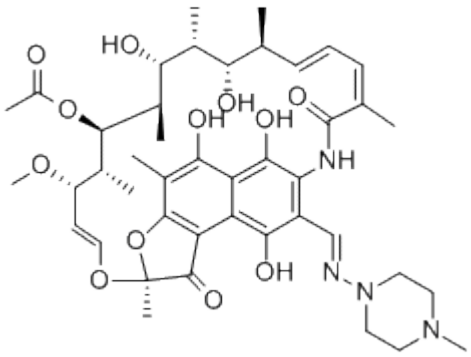

Figure S2: Structure of RIF.