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Validation of a method for measuring rifampicin plasma drug concentration in tuberculosis patients

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Abstract
Rifampicin is a first-line drug used in the management of tuberculosis (TB) due to its sterilizing effect that targets the bacteria responsible for the disease. This study modified and validated a method for quantitatively determining rifampicin plasma drug concentration of TB patients through High Performance Liquid Chromatography Ultraviolet-Visible detection. The analytic method modified from a standard method was validated for linearity, coefficient of variation, intra-day and inter-day precision, and recovery rate. The retention time of rifampicin was reduced from 18.3 to 3.1 minutes after the method was modified. To validate the method, the coefficient of determination was determined for the linearity to be between 0.975 and 0.998, the coefficient of variation ranged between 2.8% to 15.5% for both intra-day and inter-day precision. Recovery rates ranged between 87% and 125%. The results indicated that the analytical method developed was valid, precise and feasible, and could be useful for quantitatively determining rifampicin plasma drug concentration in TB patients.

Keywords: HPLC-UV/Visible; Plasma rifampicin concentration; Method development; Tuberculosis; Nigeria

INTRODUCTION

Tuberculosis (TB) is a major global health problem, being the 13th leading cause of death worldwide, and the 2nd leading cause of death from a single infectious agent after COVID-19 [1]. Of the total number of people who develop TB each year, about 90% are adults, with more cases among men than women [2]. *Mycobacterium tuberculosis* (*M.TB*), the causative agent for TB is an aerobic, non–spore-forming intracellular bacillus that replicates very slowly; therefore, a prolonged multi-drug treatment regimen of four months to one year is the recommended treatment strategy, implemented through Directly Observed Therapy (DOT). The World Health Organization (WHO) stated that TB is still highly relevant because the incidence of *M.TB* infections with increasingly complex resistance profiles has increased worldwide [1].

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Rifampicin is the major first-line drug used in the management of drug-sensitive TB due to its sterilizing effect that is bactericidal against intracellular, extracellular, and dormant microorganisms within one hour of administration [3]. Its ability to penetrate and destroy persistent intracellular organisms makes it extremely valuable in short-course chemotherapy regimens. Rifampicin is reported to be 90% readily absorbed from the gastrointestinal tract, with peak plasma concentrations of 4 to 32 µg/ml (mean value of 7 µg/ml), obtained 1.5 to 4 hours after ingestion of 600 mg dose (10 mg/kg/d) on an empty stomach [4]. About 89% of rifampicin in circulation is bound to plasma proteins. Rifampicin is stable in plasma at room temperature for up to 6 hours, making it one of the first-line anti-tuberculosis drug to be easily assayed at room temperature [4,5].

Regimens without rifampicin or in which rifampicin was only used for the first two months of therapy resulted in higher rates of tuberculosis treatment failure and relapse. There is an increasing incidence of Multi-Drug Resistant-TB (MDR-TB) and Extensive Drug Resistant - TB (XDR-TB) globally due to treatment failure and non-adherence [6]. A sub-therapeutic level, below the minimum effective concentration may also negatively affect therapeutic outcomes. Measuring rifampicin plasma drug concentration in tuberculosis patients, especially in multidrug-resistant TB patients will enable the relevant health care workers know if the concentration is within the therapeutic window (above minimum effective concentration and below toxic concentration) so that a safe and effective therapy is ensured [4]. Therefore, therapeutic drug monitoring of rifampicin implemented through High-Performance Liquid Chromatography-HPLC analysis may be useful in monitoring and evaluating therapeutic outcomes in this group of patients. Measuring drug concentration in blood plasma or serum is the most direct and commonly used approach to assess the pharmacokinetics of drugs in the body, from which the patient's level of adherence can be determined to check if the drug concentration in the body is within the therapeutic window [7].

The principle of HPLC is based on the separation of a sample into its constituent parts due to the difference in the relative affinities of different molecules for the mobile phase and the stationary phase used in the separation. Retention time is the time between the sample's injection and the maximum response for the solute's peak. The peak area can be correlated with plasma concentration. Retention times obtained from standard methods already developed may give longer times when the methods are adapted [8]. This may not be feasible especially when large numbers of samples are to be assayed, thus necessitating the need for modification of the method to obtain shorter retention times, as was the case of this study. The sensitive nature of the column requires that the method chosen for analysis be verified through method development and validation to ensure that clear separations and peaks of the drug, internal standard, and antioxidant (if needed) are obtained. Standard parameters for analytical method validation include: Coefficient of determination for linearity which is a measure of validity ranging between zero and one. The closer it is to one, the more valid the result. A value less than 0.9 invalidates the method. Coefficient of variation (CV) measures accuracy and precision obtained from intra-day and inter-day precision values. A CV value less than 20% indicates good precision [9]. This study therefore developed a valid, precise, and feasible method for quantitatively determining rifampicin plasma drug concentration in TB patients, modified from a standard method [10,11].

EXPERIMENTAL METHODS

Study design and setting. This experimental study was done to determine a feasible method for measuring rifampicin plasma drug

concentration of TB patients in Jos, Plateau State. The State is located in North-Central Nigeria and has a land mass area of 26,899 square kilometers with a population of about 3,500,000 people [12]. The study was carried out in five Directly Observed Treatment (DOT) centers for TB in Plateau State: Bingham University Teaching Hospital (BUTH) Jos, Faith Alive Hospital (FAF) Jos, and Our Lady of Apostles (OLA) Jos, which are faith-based hospitals. Plateau State Specialist Hospital (PSSH) Jos is a tertiary health institution owned by the Plateau State Government. The fifth DOT center was the Primary Health Care clinic, Tudun Wada, Jos, Plateau State. These DOT centers were chosen based on preliminary information that they account for more than 50% of reported and documented TB cases in Plateau State.

Study population and sampling method. The study population was TB patients already on first-line multi-drug DOT regimen that includes rifampicin. The patients were conveniently sampled as they came for their medication refills. They were diagnosed based on clinical symptoms and confirmed by microscopic detection of acid-fast bacilli. TB patients not on rifampicin, below 15 years of age, on rifampicin for less than two weeks for TB treatment, Multi-Drug Resistant (MDR) TB patients, and HIV/TB co-morbid patients were excluded from the study.

Ethical consideration. The Institutional Health Research Ethics Committee of the Jos University Teaching Hospital, Jos, Nigeria, with reference number: JUTH/DCS/ADM/127/XIX/6442 approved the study protocol to enable access to the data used for the research. De-identified patient data were used for analysis to ensure confidentiality of patient records. After a careful explanation of the intent of the research, informed written consent was obtained from the patients before data collection.

Data collection instruments. The materials used for the pharmacokinetic analysis were: Needle and syringe (2 ml and 5 ml), EDTA vacuum tubes, transfer pipettes, cryo vials (1.8 ml), cryo boxes, plain sample bottles, and latex hand gloves.

The reagents used were: Acetonitrile HPLC grade (Lichrosol® by Merck Germany), Anhydrous Potassium Dihydrogen Phosphate-KH_2PO_4 (JT Baker® New Jersey USA), Orthophosphoric acid (VWR Prolabo chemical, France), Reference standard rifampicin, ascorbic acid, and sulphadoxine (USP).

The equipment used included: Centrifuge (Rotofix®); Vortex Mixer (J.P. Selectra®, Spain); Sonicator (Mrc®); Weighing Balance (Shimadzu model ATX224); Filteration Unit: Pump (Charles Austen®); Solvent Filter/degasser assembly (Agilent® technologies, Germany); Micropipettes: 10-100 µl and 100-1000 µl (Socorex ACURA® 825 Swiss); Pipette tips: 2-200 µl and 100-1000 µl (Diamond® Tipack for Pipetman® Gilson); Syringe filter (0.45 µm); HPLC (Agilent® technologies, 1200 infinity series; 1260 infinity, 1260 Quaternary pump, Germany).

Preparation of standard solutions Calibration of reference standard rifampicin. Reference standard rifampicin-RF (10 mg) was dissolved in 10 ml of acetonitrile to give a 1 mg/ml solution. A 1 in 10 dilution (1 ml stock solution made up to 10 ml with acetonitrile in a volumetric flask) was done to obtain the working concentration of 100 µg/ml. Serial dilution was prepared with the plasma to obtain concentrations of 4, 6, 8, 10, 15, 20, and 30 µg/ml.

Preparation of 10 mM KH_2PO_4 mobile phase for HPLC. Anhydrous potassium dihydrogen phosphate-KH_2PO_4 (1.3609 g) was weighed and dissolved in 200 ml distilled water in a beaker. It was sonicated for one minute and transferred to a 1000 ml volumetric flask and made up to the mark with distilled

water. Orthophosphoric acid was added to adjust the pH to 5.0. Sonication was done for another one minute. The solution was filtered in the filtration unit before transferring it to the HPLC mobile phase bottle.

Preparation of standard stock solutions Ascorbic Acid-AA (antioxidant). Exactly 200 mg standard AA was dissolved in 10 ml distilled water to give a 20 mg/ml solution. **Sulphadoxine-SP.** Exactly 100 mg of standard SP (Internal Standard) was dissolved in 100 ml methanol to give a 1 mg/ml solution.

Data Collection

Blood sample collection and storage. The recruited patients did not take any food or drugs from 11 PM on the day preceding blood sample collection until four hours after intake of their anti-TB medicines that included rifampicin (10 mg/kg). Drinking water was allowed *ad libitum*. The patients were told not to consume any drinks containing alcohol, caffeine, tea, and chocolate the night preceding and the following day during drug intake and blood sample collection.

The blood samples (4 ml) collected from the eligible TB patients with 5 ml sterile needles and syringes were transferred to 5 ml vacuum EDTA tubes at times 0, 2 hours, and 4 hours after drug intake. The samples were immediately transported to the Institute of Human Virology Laboratory, Plateau State Specialist Hospital (average transportation time was six minutes), where they were centrifuged at 3000 rotations per minute for 10 minutes to enable the separation of plasma from blood cells. A transfer pipette was used to aliquot the plasma into labeled cryo vials which were placed in cryo boxes. The transfer was done in a biological safety cabinet. The plasma samples were stored at -86 °C. A total of 279 plasma samples from 93 TB patients were collected and stored. Blank plasma (60 ml) from a screened healthy volunteer was obtained from the blood bank of Plateau State Specialist Hospital. It was transferred into cryo vials in the biosafety cabinet, kept in cryo boxes, and stored in the -86 °C freezer. The frozen plasma was transported by airplane in a cooler padded with ice packs from Jos, Plateau State to Lagos State, Nigeria for about three hours, and transferred to a -80 °C freezer in the AIDS Prevention Initiative Nigeria (APIN) laboratory of the College of Medicine of University of Lagos, Lagos University Teaching Hospital (LUTH).

Extraction of rifampicin from plasma.

As per the standard method, 250 µl of acetonitrile + 25 µl ascorbic acid solution (20 mg/ml) + 100 µl sulphadoxine (1 mg/ml) were added to 500 µl of plasma sample from a TB patient, vortexed for 20 seconds and centrifuged for five minutes at 3000 rotations per minute (rpm). The buffer (200 µl of KH_2PO_4) was added, the mixture was vortexed for 20 seconds and centrifuged for five minutes at 3000 rpm. The supernatant was withdrawn, filtered through a 0.45 µm syringe filter into HPLC vials, and fed to the auto-sampler unit of the HPLC at an injection volume of 50 µl. The wavelength for UV detection was 334 nm [11].

A. The standard method was initially adapted as follows: A 200 µl quantity of blank plasma**,** 100 µl acetonitrile, and 10 µl ascorbic acid solution (20 mg/ml) were added to 100 µg/ml of reference standard rifampicin (USP), vortexed for 20 seconds, and centrifuged for five minutes at 3000 rotations per minute (rpm). A buffer (200 µl of KH_2PO_4) was added, vortexed for 20 seconds, centrifuged for five minutes at 3000 rpm. The supernatant was withdrawn and 50 µl was injected into the HPLC. The chromatographic conditions were set at: Column: Zorbax extend - C18 analytical, 250 x 4.6 mm, 5 – micron 80Å; Column temperature: 30 °C; Mobile phase: ACN: 10 mM KH_2PO_4 (38%:62%), pH 5.0; UV wavelength: 334 nm; Flow rate: 1 ml/min. This gave 18.30 minutes of retention time for rifampicin (Figure 1). Blank plasma (200 µl) with 10 µl ascorbic acid gave a retention time of 1.97 minutes for ascorbic acid. The retention

time of 18.3 minutes per sample obtained was not feasible given that there were 279 samples to be analysed. Therefore, a step-wise modification of the method was done as shown below:

Modified analytical method development.

B. Rifampicin (6 µg/ml) in plasma was injected into the HPLC under the same conditions as A above but a 20 µl injection volume gave a retention time of 16.01 minutes.

C. Rifampicin (6 µg/ml), under the same conditions above with an increase in flow rate from 1 ml/min to 1.5 ml/min reduced the retention time to 11.05 minutes.

D. The mobile phase ratio when changed from 38:62 to 40:60 ACN and buffer respectively at 1.5 ml/min flow rate gave a retention time of 8.19 minutes.

E. The mobile phase ratio was modified again from 40:60 to 42:58 ACN and buffer respectively to give a retention time of 6.08 minutes (Figure 2).

F. Hydrochlorothiazide - HCT (6 µg/ml) reference standard (USP) was initially used as the internal standard but its retention time of 1.83 minutes was too close to that of ascorbic acid-AA (1.97 minutes). When repeated, 1.83 and 1.56 minutes HCT and AA retention times were obtained respectively. When injected with rifampicin, the retention times for AA, HCT, and RF were 1.56, 1.83, and 5.98 minutes respectively.

G. Sulphadoxine - SP (20 µg/ml) reference standard (USP) injected alone after washing gave a longer retention time of 2.38 minutes. When combined with rifampicin, the retention times were 2.38 and 5.79 minutes respectively. Thus, sulphadoxine was chosen as the internal standard instead of hydrochlorothiazide.

H. AA + 10 µg/ml SP + 10 µg/ml RF gave retention times of 1.296, 2.21, and 3.06 minutes respectively (Figure 3).

I. A tuberculosis patient's plasma sample was picked at random, subjected to the same conditions in H above and retention times of 1.30, 2.24, and 3.05 minutes for ascorbic acid, sulphadoxine, and rifampicin respectively were obtained (Figure 4).

Modified analytical method. In the modified method, 250 µl of acetonitrile, 25 µl ascorbic acid solution (20 mg/ml), 100 µl sulphadoxine (1 mg/ml) were added to 500 µl of plasma sample from a TB patient, vortexed for 20 seconds, and centrifuged for five minutes at 3000 rpm. The buffer (200 µl of KH_2PO_4) was added, vortexed for 20 seconds, and centrifuged for five minutes at 3000 rpm. The supernatant was withdrawn, filtered through a 0.45 µm syringe filter into HPLC vials, and feed to the auto sampler unit of the HPLC with an injection volume of 20 µl. The chromatographic analysis was performed at ambient temperature using Agilent® 1260 infinity series (Agilent Technologies, Germany) HPLC, coupled with an auto sampler and Ultraviolet-Visible detector. The detector output was linked to a HP Compaq LA1951 configured to Chem-Station Chromatography software. The computer system was connected to an HP LaserJet P3015 printer. The column was Zorbax extend® C-18 analytical 250 × 4.6 mm, Internal Diameter x 5µm, 80 Å, Agilent. The column temperature was maintained at 30 oC. The mobile phase consisted of Acetonitrile: Phosphate Buffer (42:58) adjusted to pH 5.0 with orthophosphoric acid. The mobile phase was filtered in the filtration unit and pumped through the column at a flow rate of 1.5 ml/min. The isocratic method was used. The detector wavelength was set at 334 nm.

Analytical method validation. The modified analytical method developed was validated using coefficient of determination for linearity, Coefficient of variation, precision, and recovery rate (RR), which are standard analytical validation parameters [9,11,13]. The linearity was done using plasma concentrations of 8, 10, 15, 20, 25, and 30 µg/ml of rifampicin and injected twice each into the HPLC to obtain peak areas. The peak area ratios of

rifampicin in ascorbic acid for the varying concentrations were calculated in the presence of sulphadoxine as the internal standard. The peak area ratio was plotted against concentration to obtain the regression equation. The coefficient of determination (R2), which assessed the linearity was obtained from the calibration plot. The R2 was calculated and evaluated based on the accepted value of > greater than 0.9 [10].

The intra-day precision was done twice per day using 4, 10, and 20 µg/ml of plasma samples, while the inter-day precision was done each day for three consecutive days using 8, 10, and 20 µg/ml to calculate the coefficient of variation. The intra-day and inter-day precision were repeated a week later to account for sample stability using 4, 10, and 20 µg/ml of rifampicin and sulphadoxine internal standard injected six times each into the HPLC to obtain the average peak area ratios for each concentration, from which the coefficient of variation was calculated. The recovery rate was evaluated by preparing rifampicin standard concentration in plasma and acetonitrile in the presence of the internal standard. The coefficient of variation and recovery rate were calculated using the equations below.

$$\text{Coefficient of Variation (CV)} = \frac{\text{Standard Deviation}}{\text{Mean}} \times 100$$

$$\text{Recovery Rate (RR)} = \frac{\text{Peak Area Ratio extracted in Plasma}}{\text{Peak Area Ratio in Blank Solvent}} \times 100$$

RESULTS

Patient characteristics. Majority of the patients were males (69%), single (52%), with secondary level of education (57%), within the reproductive age of 15-44 years, and employed in private and government organizations (61%). About 22% had comorbid illnesses such as hypertension, diabetes mellitus, ulcer, arthritis, and cancer as shown in Table 1.

Analytical method development. The analytical method used in this study gave an initial rifampicin retention time of 18.3 minutes (Figure 1), which was not feasible given that there were 279 samples to be analyzed. This led to the modification of the method in successive stages (B - I) until a retention time of 3.1 minutes was obtained for rifampicin, with clear separated retention times for the internal standard (sulphadoxine) -2.2 minutes and the antioxidant (ascorbic acid) -1.3 minutes as seen in Table 2 and Figures 3 and 4.

Validation of modified analytical method developed. The coefficient of determination values were above 0.9, the coefficient of variation was below 20%, and recovery rates were above 70% for all stages of the method development as shown in Tables 3 - 5 and Figure 5.

DISCUSSION

A valid, precise, and feasible analytical method was developed for the quantitative determination of rifampicin plasma drug concentration in TB patients. A retention time of 3.1 minutes for rifampicin was obtained from the modified analytic method developed, 2.2 minutes for the internal standard (sulphadoxine), and 1.3 minutes for the antioxidant (ascorbic acid). The modified method developed was validated using coefficient of determination for linearity, coefficient of variation, intra-day and inter-day precision, and recovery rate (RR) which are standard parameters as in other literature reports [9,11,13].

The linearity results presented in Tables 3 and 4, and Figure 5 indicated a high correlation between peak area and rifampicin drug concentration, with the coefficient of determination (R2) greater than 0.9. The R2 obtained from this study ranged between 0.97 and 0.99, implying that the peak area was equivalent to concentration as similarly observed in previous studies [6,9,10] where R2 values above 0.95 were obtained. R2 is a measure of linearity that shows the degree of

relationship between concentration and peak area. The closer it is to one, the more valid the result. A value less than 0.9 invalidates the method because peak area should be highly correlated with concentration [10].

The coefficient of variation measures the degree of precision of the method. Values ranging from 7.6% to 15.5% were obtained from this study. A value less than 20% indicates good precision. Once a method is valid and precise, it can be generalized [13].

Table 1: Tuberculosis patient characteristics n=93

Variable	Category	Frequency (%)
Age	15-24	26 (28.0)
	25-34	23 (24.7)
	35-44	27 (29.0)
	45-54	10 (10.8)
	>54	7 (7.50)
Sex	Male	64 (68.8)
	Female	29 (31.2)
Occupation	Employed	57 (61.3)
	Housewife	6 (6.5)
	Student/Applicant	27 (29.0)
	Others*	3 (3.3)
Educational Level	No formal Education	11 (11.8)
	Primary	14 (15.1)
	Secondary	53 (57.0)
	Tertiary	15 (16.1)
Marital Status	Single	48 (51.6)
	Married	42 (45.2)
	Divorced	1 (1.1)
	Widow/widower	2 (2.2)
Phase of Treatment	Intensive	44 (47.3)
	Continuation	49 (52.7)
Alcohol Intake History	Yes	2 (2.2)
	No	67 (72.0)
	No but positive previous history	24 (25.8)
Cigarette Smoking History	No	78 (83.9)
	No but positive previous history	15 (16.1)
Co-morbidity**	Yes	20 (21.5)
	No	73 (78.5)

Others*=Retired, Farmer; Co-morbidity**=Hypertension, Diabetes Mellitus, Arthritis, Peptic ulcer

Table 2: Analytical Method Development

	Concentration (µg/ml)	Injection volume (µl)	Flow rate (ml/min)	ACN:KH_2PO_4 Buffer (%)	Retention time (min)		
					RF	IS	AA
A	100 RF stock	50	1	38:62	18.3		
B	6 RF	20	1	38:62	16.0		
C	6 RF	20	1.5	38:62	11.1		1.3
D	6 RF	20	1.5	40:60	8.2		1.3
E	6 RF	20	1.5	42:58	6.1		1.3
F	6 RF + 6 HCT	20	1.5	42:58	6.0	1.8	1.5
G	6 RF + 20 SP	20	1.5	42:58	5.8	2.4	1.3
H	10 RF +10 SP	20	1.5	42:58	3.1	2.2	1.3
I	RF + 10 SP (random TB patient plasma)	20	1.5	42:58	3.1	2.2	1.3

AA=Ascorbic Acid; ACN=Acetonitrile; HCT=Hydrochlorothiazide; IS=Internal Standard; RF=Rifampicin; SP=Sulphadoxine

Table 3: Linearity data

Conc. µg/ml	A RF alone	B RF+AA	C RF+PL+IS+AA	D RF+ACN+IS+AA	C/D×100
	Av. Peak Area	Peak Area	Peak Area Ratio (RF/IS)	Peak Area Ratio (PAR)	Recovery Rate (%)
4			1.350	1.080	125.00
6		171.670	1.810	1.780	101.70
8	92.360	250.450	2.090	2.380	87.80
10	111.860	302.190	2.650	2.960	89.60
15	172.220	450.660	4.310	4.190	102.86
20	241.950	593.920		6.200	
25	307.980				
30	369.360				
R^2	0.995	0.998	0.975	0.991	

AA=Ascorbic Acid, ACN=Acetonitrile, Av. =Average, Conc. =Concentration, IS=Internal Standard, PL=Plasma, R2=Coefficient of Determination, RF=Rifampicin, Recovery Rate=Peak Area Ratio-plasma(C)/PAR blank solvent (D) x 100).

Table 4: Intra-day Precision

Conc. µg/ml	Peak Area RF	Peak Area IS	PAR	Av. PAR	SD	CV (%)	R^2
4	114.50	194.70	0.59	0.61	0.0172	2.8	0.9960
	119.04	188.31	0.63				
	116.41	188.15	0.62				
	112.80	188.85	0.60				
	112.70	189.52	0.59				
	114.51	191.36	0.60				
10	283.91	189.22	1.50	1.48	0.0116	0.8	
	282.09	189.04	1.49				
	283.63	191.38	1.48				
	285.59	192.80	1.48				
	286.10	193.90	1.48				
	286.92	195.46	1.47				
20	562.49	191.20	2.94	2.79	0.3055	10.9	
	557.49	190.09	2.93				
	558.66	191.94	2.91				
	557.03	192.26	2.90				
	543.26	250.73	2.17				
	568.69	197.29	2.88				

Av. =Average, Conc.= Concentration, CV= Coefficient of Variation, IS=Internal Standard, PA=Peak Area, PAR=Peak Area Ratio, RF=Rifampicin, R2=Coefficient of Determination, SD=Standard Deviation.

Table 5: Inter-Day Precision

Conc. µg/ml	Day 1 PA	Day 2 PA	Day 3 PA	Day 4 PA	MEAN PA	SD	CV (%)
8	229.65	232.13	171.67	250.45	220.98	34.15	15.50
10	279.95	250.45	279.35	302.19	277.99	21.21	7.60
20	604.60	605.15	450.66	593.92	563.58	74.46	13.40

CV= Coefficient of Variation (SD/mean x 100), PA=Peak Area, SD=Standard Deviation

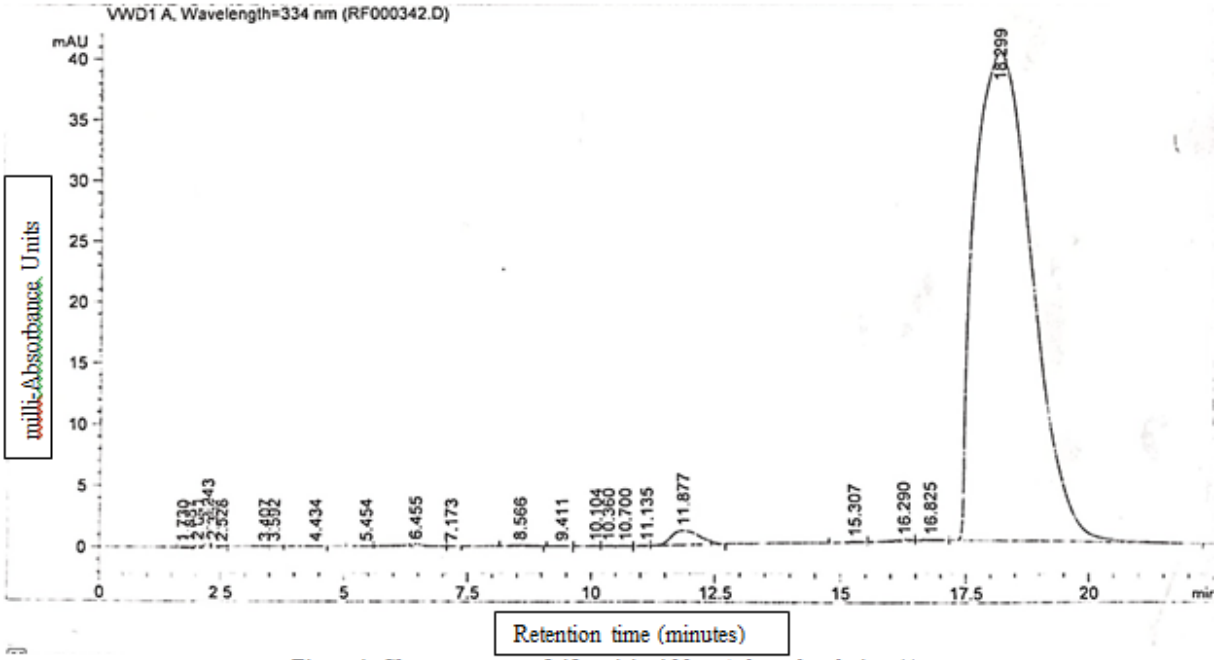

Figure 1. Chromatogram of rifampicin 100 µg/ml stock solution (A)

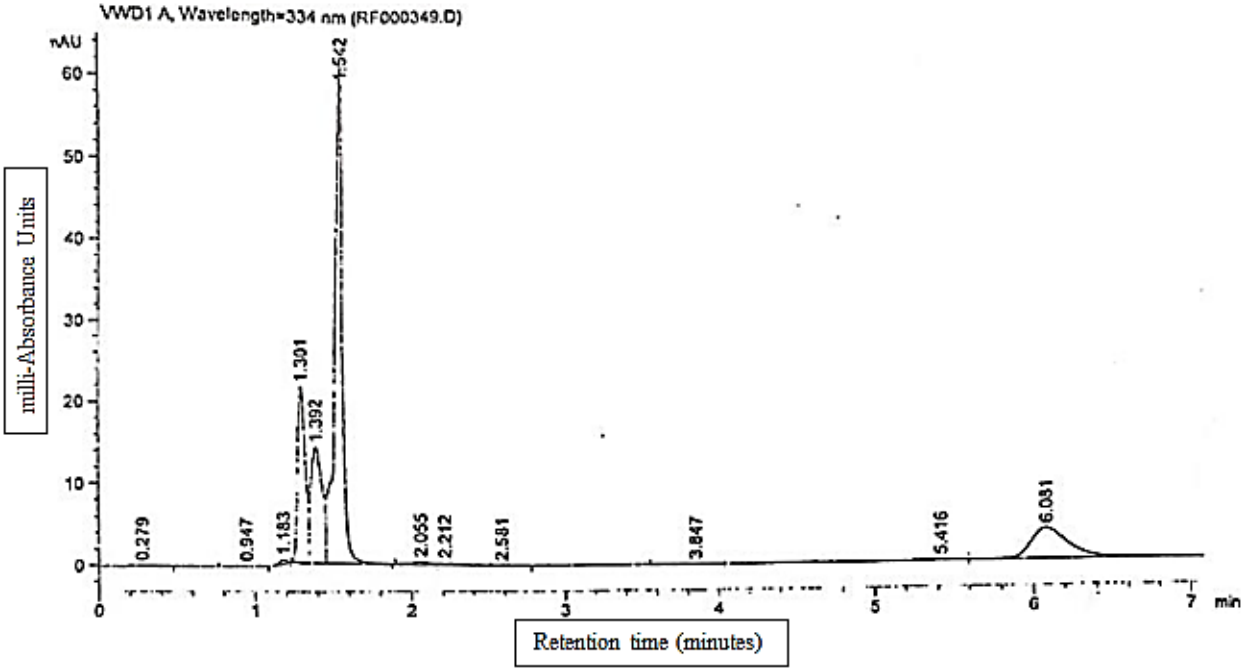

Figure 2. Chromatogram of rifampicin 6 µg/ml with acetonitrile:KH2PO4 buffer 42:58 (E)

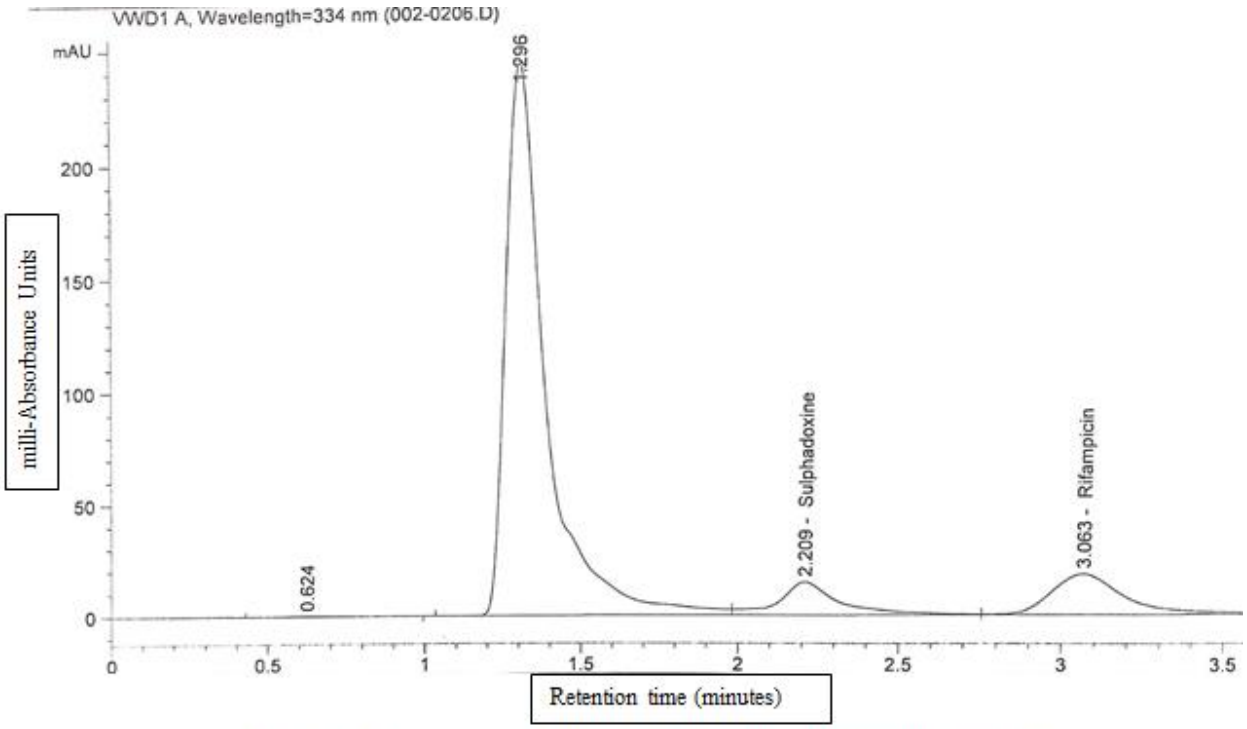

Figure 3. Chromatogram of rifampicin 10 µg/ml and 10 µg/ml sulphadoxine (H)

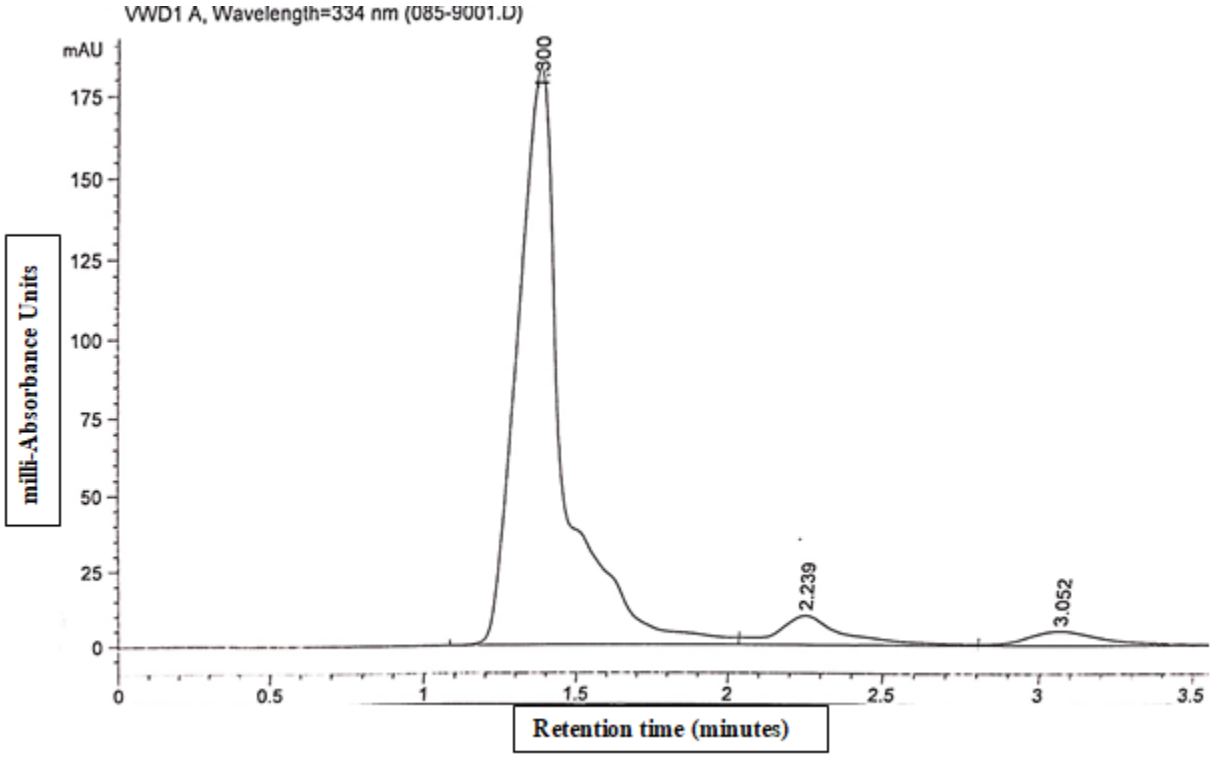

Figure 4. Chromatogram of rifampicin in random tuberculosis patient's plasma (I)

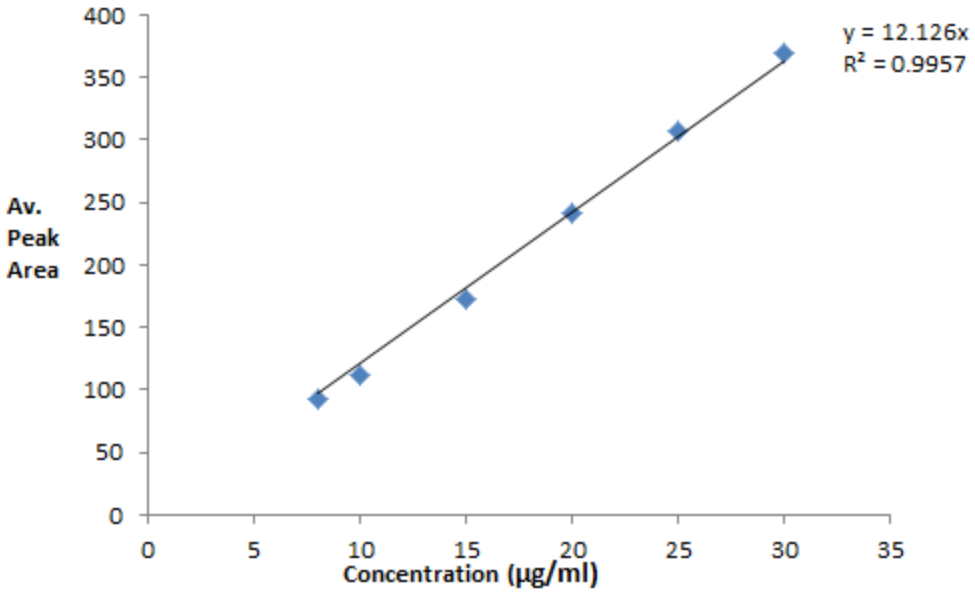

Figure 5. Calibration plot of average peak area vs. rifampicin concentration in plasma (Intra-day precision) R^2 = *Coefficient of Determination*

The recovery rate determines the percentage of drug recovered from plasma that was available for quantification which should be greater than 70% [13,14]. Values ranging from 87% to 125% were obtained in this study, indicating high recovery of rifampicin and implying that the extraction method used was adequate and can be reproducible [7]. In addition, data obtained from the intra-day and inter-day precision determination indicates that the assay method is reproducible within the same day and on different days [15]. The coefficients of determination and variation obtained from this study indicated that the modified method was valid, precise, and can be used in measuring rifampicin plasma drug concentration in TB patients.

Clinically, the use of assay procedures for determination of rifampicin plasma drug concentration and the interpretation and application of the resulting concentration data can be used to develop safe and effective drug regimens. The process allows for the achievement of therapeutic concentrations of a drug more rapidly and safely than can be attained with empiric dose changes [16].

Conclusion. In summary, a method for measuring rifampicin plasma drug concentration in TB patients was developed through the modification of a standard method and its' validation was done. In the developed and validated method, the retention time of rifampicin was reduced from 18.3 minutes to 3.1 minutes. The linearity of greater than 0.90 showed a high correlation between peak area and rifampicin plasma drug concentration. The extraction of rifampicin from plasma was adequate and reproducible with recovery rates above 70%. This indicates that the analytical method developed was valid, precise, and feasible.

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