Method Validation of an Ultra-High-Performance Liquid Chromatography (UHPLC) for the Bioequivalence Study of Rifampicin

Allen Jasper D. Diño, RCh,[1]* Essel N. Tolosa, RPh,[2]* Ailyn M. Yabes, MScPH, DrPH,[1,3] Noel S. Quiming, RPh, MS, DrEng,[4] Leonila A. Estole-Casanova, MD, MSc,[1,3] Ma. Stephanie Fay S. Cagayan, MD, PhD[3] and Cecilia A. Jimeno, MD[3]

[1]Bioanalytical and Toxicology Laboratory, Department of Pharmacology and Toxicology, College of Medicine, University of the Philippines Manila
[2]Institute of Herbal Medicine, National Institutes of Health, University of the Philippines Manila
[3]Department of Pharmacology and Toxicology, College of Medicine, University of the Philippines, Manila
[4]Department of Physical Sciences and Mathematics, College of Arts and Sciences, University of the Philippines Manila

ABSTRACT

Objectives. In response to the need for a simple and fast way of ensuring that generic drugs especially those that contain rifampicin are bioequivalent with reference drugs, this study validated an ultra-high-performance liquid chromatography (UHPLC) method of quantifying rifampicin in human plasma. The study also validated the method's selectivity, linearity, sensitivity, accuracy, precision, and the absence of a carry-over effect adhering to the Philippine Food and Drug Administration guidelines.

Methods. Plasma samples were prepared via protein precipitation using methanol containing ascorbic acid. Three microliters (3 uL) of the prepared samples were then analyzed in a Waters Acquity H-Class UPLC® system coupled to a tunable ultraviolet (TUV) detector with an attached UPLC® BEH C-18 column using a developed and optimized method. Briefly, the column temperature was set to 40°C and the sample temperature was set to 10°C. Elution was done using a linear gradient flow of a water-acetonitrile mixture that started with 45% acetonitrile increasing to 60% acetonitrile at 0.5 minutes and back to 45% acetonitrile at 3 minutes and having a constant flow rate of 0.5 mL/min. Detection was done at 340 nm. Method validation was performed following the ICH guidelines for Bioanalytical Method Validation, the same guidelines referenced by the ASEAN Guideline for Harmonisation of Standards and the Philippine Food and Drug Administration (FDA).

Results. The method had an analysis time of 3 minutes wherein rifampicin eluted at 1.4 minutes while the internal standard, rifapentine (IS) eluted at 1.7 minutes. Since no co-eluting endogenous materials were observed for the rifampicin and the internal standard, the method was confirmed to be selective. Its linearity over the range of 2 ug/mL to 25 ug/mL has been validated where it has a limit of detection (LOD) and limit of quantification (LOQ) values of 0.64 ug/mL and 1.94 ug/mL, respectively. The interday and intraday precision, reported as % coefficient of variance (%CV), and interday and intraday accuracy, reported as %error all within the limits of ±20% for the LLOQ and ±15% for the rest indicating its reliability and reproducibility. Lastly, due to the nature of the injection of the sample into the system, wherein a blank immediately follows the highest concentration standard, the method has been cleared of a carry-over effect.

Conclusion. The study successfully validated a UHPLC method of quantifying rifampicin in human plasma. Due to the sample processing method used and the chromatographic conditions set, the method can prepare and analyze samples in a simple yet fast, sensitive, reliable, and reproducible manner. The method can be applied in bioavailability and bioequivalence studies of rifampicin.

Keywords: rifampicin, bioequivalence, UHPLC, validation

* Mr. Diño and Mr. Tolosa shared first authorship for this manuscript.

INTRODUCTION

Rifampicin is a first-line drug prescribed for the treatment of tuberculosis (TB). It is often taken with other antibiotics such as pyrazinamide, isoniazid, and ethambutol in a fixed-dose combination (FDC) manner. The development of these FDCs became the cornerstone of TB treatment as it offered a simpler treatment regimen, better patient adherence, and reduced risk of the emergence of drug-resistant strains and medication errors.[1-4] However, despite the efficacy of FDCs, concerns were raised when studies discovered a problem with rifampicin that could compromise the overall effectiveness of the treatment. Owing to its physicochemical properties, the effect of excipients during formulation, and the effect of co-administered drugs in FDCs, rifampicin has been observed to exhibit variable bioavailability resulting in reduced plasma concentrations sometimes even going below the therapeutic threshold.[2,5-7] Thus, since treatment formulation using rifampicin remains effective, the World Health Organization (WHO) and the International Union Against Tuberculosis and Lung Diseases (IUATLD) recommended that drugs containing rifampicin should only be used and put into the market once an acceptable bioavailability and bioequivalence (BA/BE) studies of at least the rifampicin component have been established.[3,8]

To date, liquid chromatography-mass spectrometry (LC/MS) and high-performance liquid chromatography-ultraviolet (HPLC-UV) have been the go-to systems for doing BA/BE studies.[4,9-14] An HPLC or UHPLC coupled to a mass spectrometer detector offers fast and sensitive analytical runs. However, because the system is fairly complex and requires technical expertise to use, in addition to the expensive cost of acquisition and maintenance, due to the MS detector, its application in the industry and research laboratories for high-throughput processing has its limitations. On the other hand, the HPLC-UV system offers a cost-effective and easy-to-use system, however, it also has its limitations brought about by the relatively lower sensitivity and longer analytical runs. Hence, there is a need for a fast, sensitive, reliable yet cost-effective method of quantifying rifampicin in human plasma.

The study validated an ultra-high-performance liquid chromatography (UHPLC) assay of measuring rifampicin in human plasma in response to the need for a simple and efficient method of ensuring that the drugs containing rifampicin are bioequivalence with reference drugs. Using this, the problem with the cost-effectiveness of LC/MS-based methods and the problem with the sensitivity and lengthy run time of HPLC-based methods are both addressed. Additionally, the UHPLC method has also been validated using the ICH guideline for Bioanalytical Method Validation (ICH guideline M10) which is referenced both by the ASEAN Guidelines for Harmonisation of Standards as well as the Philippine FDA.[15] These guidelines ensure global harmonization in developing and registering safe, effective, and high-quality medicines.[15] The validated method will serve as a prerequisite in the establishment of a bioavailability/bioequivalence unit at the Department of Pharmacology and Toxicology, College of Medicine, University of the Philippines Manila.

METHODS

Solvents and Reagents

All solvents used were liquid chromatography grade. Water (LC-MS grade) was purchased from Duksan Pure Chemicals Co. Ltd while methanol (HPLC grade) and acetonitrile (HPLC grade) were both purchased from Scharlab Philippines Inc. Pharmaceutical grade rifampicin and rifapentine as well as AR grade ascorbic acid were all purchased from Sigma-Aldrich Pte Ltd. Drug-free stocks of human plasma were obtained from the Philippine General Hospital Blood Bank.

Instrumentation and Chromatographic Conditions

A Waters Acquity H-Class UPLC® System was used in this study. The system was equipped with a TUV Detector, Sample Manager-FTN, and a Quaternary Solvent Manager, all controlled using the EMPOWER 3 Software. Separation of the rifampicin and the internal standard (IS) detected at 340 nm was conducted on an Acquity UPLC® BEH C-18 column (1.7 um, 2.1 mm x 50 mm i.d.) at 40°C using a solvent system composed of HPLC Grade Acetonitrile (0.1% TFA; Solvent B) and LC-MS Grade Water (0.1% TFA; Solvent A) delivered in gradient elution. The total run time for the analysis was 3 minutes. The chromatographic conditions developed and optimized for the system are shown in Table 1.

Preparation of Standard Solutions, Calibration Controls, and Quality Controls

Stock solutions of rifampicin and rifapentine (internal standard) at 1000 μg/mL each were prepared in methanol containing 1000 μg/mL of ascorbic acid. Internal standards (IS) are similar to the sample but not identical and should not be present in the sample matrix. Rifapentine was used as an internal standard to ensure accuracy and precision by compensating various potential sources of errors in the

Table 1. Developed UPLC Elution Program

Solvent A	LC-MS Grade Water (0.1% TFA)			
Solvent B	HPLC Grade Acetonitrile (0.1% TFA)			
Column Temp	40°C			
Autosampler Temp	10°C			
Injection Volume	3 uL			
Gradient	Time (min)	Flow Rate (mL/min)	% A	% B
	0.00	0.500	55.0	45.0
	0.50	0.500	40.0	60.0
	3.00	0.500	55.0	45.0

analysis. Ascorbic acid was added to prevent degradation of the standards via oxidation. Two batches of working solutions of rifampicin at 50 ug/mL, 100 ug/mL, and 250 ug/mL, and one batch of rifapentine at 375 ug/mL, were prepared in amber-colored volumetric flasks by taking an amount from the respective stock solution and diluting them with methanol containing 10 ug/mL of ascorbic acid. Before use, all these solutions were stored in a -20°C freezer away from direct light.

The first batch of rifampicin working solutions was used to prepare the 6 concentration points of the calibration curve. Briefly, the working solutions were used to spike 500 uL of drug-free plasma to obtain 2 ug/mL, 5 ug/mL 10 ug/mL 15 ug/mL 20 ug/mL, and 25 ug/mL solutions. Each spiked solution (400 uL) was put into individual conical tubes and a working internal standard (100 uL) was added to each tube.

The second rifampicin working solutions were used to prepare the quality control samples. Similarly, 5 replicates of the quality control samples at 2 ug/mL, designated as the lower limit quality control (LLQC), 4 ug/mL, designated as the low quality control (LQC), 12 ug/mL designated as the medium quality control (MQC), and 25 ug/mL, designated as the high quality control (HQC) were also prepared.

Sample Extraction

Samples were prepared via protein precipitation using methanol containing 500 ug/mL of ascorbic acid. The methanol was added to the spiked plasma in a 4:1 ratio. The resulting solution was then subjected to vortex mixing for 10 seconds and loaded in a refrigerated centrifuge with conditions set to 4°C at 4000 rpm. This ran for 15 minutes. After centrifugation, 1 mL of the supernatant was obtained and filtered with a 0.45 μm syringe filter wherein 3 μL of the filtered solution was injected into the system.

Method Validation

The method was validated following the ICH guideline for bioanalytical method validation (ICH Guideline M10 on Bioanalytical Method Validation) which is also referenced by the ASEAN Harmonisation and the Philippine FDA.[15]

Selectivity

To determine the selectivity of the method, the chromatogram of six blank plasma samples pooled together, and the chromatograms of samples spiked with rifampicin and internal standard were compared. The method was considered selective if there were no significant peaks in the retention times of rifampicin or rifapentine (IS) in the blank plasma samples.

Linearity

The linearity of the method reflects the direct relationship between the test results and the concentration of the analytes. It is evaluated by generating a calibration curve having a range that encompasses the expected concentration of the analyte. The linearity of the method was evaluated over the calibration range of 2 μg/mL, 5 μg/mL, 10 μg/mL, 15 μg/mL, 20 μg/mL, and 25 μg/mL of rifampicin. Freshly prepared calibration curves were analyzed for five days. A plot of the ratio of the peak area of rifampicin to that of the IS versus the nominal concentration of the calibration points was generated and analyzed using linear regression. Per ICH criteria, the calibration curve is valid provided that the back-calculated value of the LLOQ is within ±20% while the rest is within ±15% accuracy.[15]

Sensitivity

Based on the guidelines, various methods can be used to present a bioanalytical method's sensitivity. For this study, the Limit of Detection (LOD) and the limit of quantitation (LOQ) was determined using the calibration curve generated. The sensitivity of the method reported as the LOQ and LOD were computed using the standard deviation from the generated calibration curve and the slope of the calibration curve as shown in equations 1 and 2 below.

$$LLOQ = \frac{10\sigma}{S} \quad \textbf{Equation 1} \qquad LOD = \frac{3\sigma}{S} \quad \textbf{Equation 2}$$

Precision and Accuracy

The intraday and interday precision and accuracy of the method were also evaluated by measuring 5 replicates of freshly prepared quality control samples. Intraday studies analysis was done on the same day in a single run while for interday studies analysis was done over five days. Precision was reported as %CV while accuracy was reported as %error. Per ICH guidelines, precision should be within ±15% for all samples. Accuracy should fall within ±15% except for the LLQC which should be within ±20%.[15]

Carry-over

To determine whether or not the method is susceptible to carry-over contaminations, the sample injection was designed such that a blank will always be injected after the highest concentration sample. The absence of a peak in the blank entailed the absence of a carry-over effect.

Ethical Considerations

The study was registered with the University of the Philippines Manila Research Grant and Administration Office (RGAO-2017-0626). Subsequently, the Institutional Biosafety and Biosecurity Committee (IBBC 2020-001) and the Research Ethics Review Board 2018-272-01 reviewed and approved this research.

RESULTS

The method validated was able to elute rifampicin and the internal standard at 1.41 minutes and 1.72 minutes, respectively (Figure 1).

Selectivity

To determine this method's selectivity, six blank plasma samples were analyzed. Its selectivity was established since no coeluting peaks were observed at the retention times of both the analyte and the internal standard as observed in Figure 2.

Linearity, Sensitivity, Precision and Accuracy

Back-calculated values of the calibration points are shown in Table 2.

The coefficient of determination (r^2) for the calibration curve was 0.9996. Following Equation 1 and Equation 2 shown in the methodology section, the LOD and LOQ

Table 2. Calculated Values of the Calibration Standards

Nominal Rifampicin Concentration	Mean Back Calculated Value (ug/mL)	%Error	%CV
2	2.157	7.853%	6.93%
5	4.931	1.373%	5.87%
10	9.822	1.783%	3.92%
15	15.138	0.092%	4.81%
20	19.779	1.103%	3.97%
25	25.149	0.060%	2.47%

of the method were computed to be 0.64 ug/mL and 1.94 ug/mL, respectively.

DISCUSSION

To ensure that rifampicin-containing medicines available in the market can deliver their intended therapeutic effect, a simple, fast, and reliable method to monitor rifampicin concentration in plasma is needed. In this study, a UHPLC-UV method was validated for the analysis of rifampicin in human plasma. The results showed that the method was within the criteria established by the ICH for bioanalytical methods.[15]

Method Validation

Validation of the method is important because it ensures the acceptability and reliability of the results being produced. The method parameters that were validated were selectivity, linearity, sensitivity, accuracy, precision, and carry-over effects.

Selectivity

In the study, selectivity was established by analyzing six different blank plasma pooled samples. The absence of any peaks at the retention times of rifampicin or rifapentine (IS)
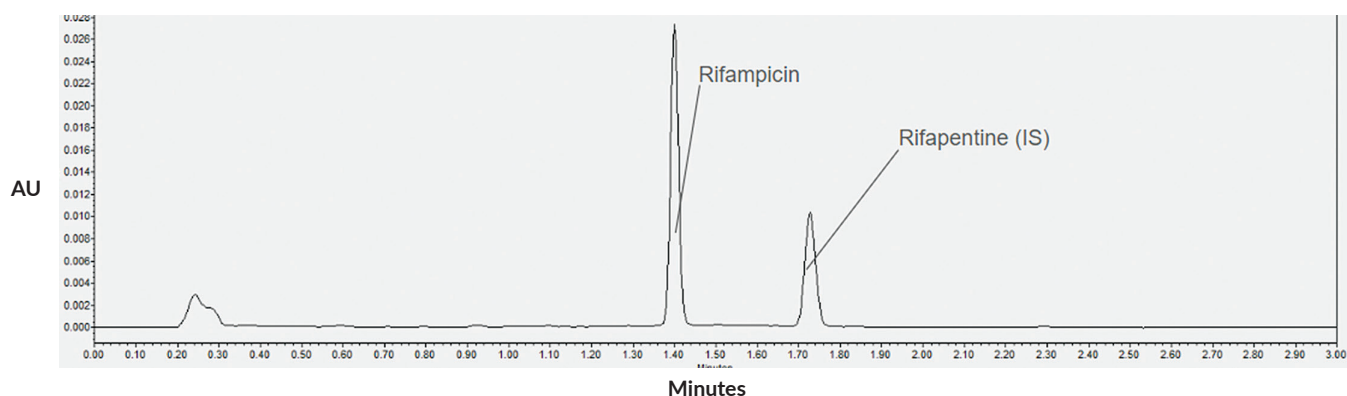

Figure 1. Sample chromatogram showing the separation of rifampicin and the internal standard, Rifapentine. Rifampicin (25 µg/mL) eluted at 1.41 minutes while Rifapentine (15 µg/mL) eluted at 1.71 minutes.
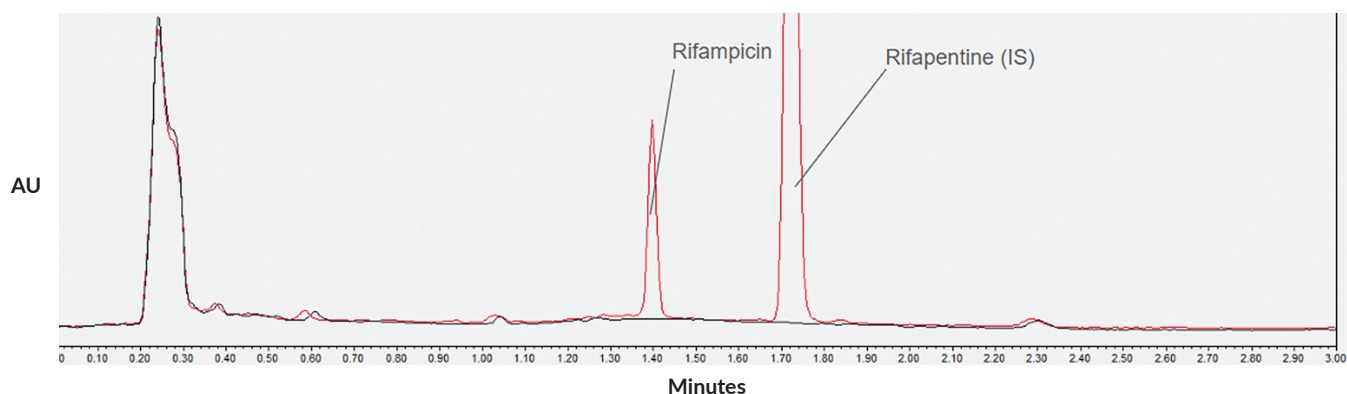

Figure 2. Overlayed chromatogram of blank pooled plasma from six sources *(black)* and the analyte 5 ug/mL rifampicin and 15 ug/mL internal standard, rifapentine *(red)*.

confirmed that the method is selective and does not suffer from interference due to plasma matrix or other endogenous substances. The absence of relevant peaks in the blank samples further confirmed the method's selectivity.

Linearity

To test the linearity, the calibration curve was generated using the rifampicin concentrations of 2, 5, 10, 15, 20, and 25 ug/mL, which covers the expected plasma concentration range following the standard dosing of rifampicin. The coefficient of determination (r^2) was 0.9996, which is within the ICH acceptance criterion of ≥0.99. Furthermore, the mean back-calculated concentrations for all calibration points being all lower than 15% established the linearity of the method.

Sensitivity

The ICH guidelines suggest that the LOD should be calculated based on the standard deviation of the response and the slope of the calibration curve while the LOQ should represent the lowest concentration that can be quantitatively determined within acceptable precision and accuracy. The computed LOD (0.64 ug/mL) and LOQ (1.94 ug/mL) of the method are significantly lower than the expected therapeutic plasma concentration of rifampicin (8-24 ug/mL), indicating the method is sufficiently sensitive. These results are within the ICH criteria, which requires the LOQ to be at least 10 times the standard deviation of the blank and the LOD should be 3 times the standard deviation of the blank.

Precision

To assess the precision of the method, both intraday and interday were evaluated. As stated in the ICH guidelines, the precision should be expressed as the coefficient of variation (%CV). The interday and intraday precision for all quality control samples (LLQC, LQC, MQC, and HQC) were within the ±20% criteria for the LLQC quality control sample and ±15% criteria for the rest of the quality control samples (Table 3). The findings confirm that the method is both precise and reproducible.

Accuracy

Accuracy is typically expressed as %recovery or % error relative to the known concentration. In this study, the accuracy was assessed using quality control samples at four different concentration levels (LLQC, LQC, MQC, and HQC). The accuracy for all quality control samples was within the ±20% limit (for LLQC) and ±15% criterion for the other quality control samples (LQC, MQC, HQC). The results demonstrated that the method is accurate according to ICH guidelines with the %error for all quality control samples remaining within acceptable limits.[15]

Carry-over

The carry-over effect is one of the most common issues reported when analyzing rifampicin in human plasma. The ICH guidelines recommend that carry-over should not interfere with the accuracy of the subsequent sample measurement.[15] To check for the carry-over effect, a blank sample was injected immediately following the highest standard/sample concentration. There were no relevant peaks detected at the retention times of rifampicin and rifapentine(IS) indicating that there was no carry-over effect.

Comparison with Other Methods

In this study, a UHPLC-UV method was chosen since it offers a faster and more sensitive analysis than what HPLC-UV systems offer but also at a much lower overall cost as compared to what LC-MS systems can do.[16-18] During the validation of the method, it was kept in mind to keep it as simple as possible without compromising the quality of the results. A non-buffered mobile phase was chosen due to the simplicity of the preparation. Preliminary experiments comparing different ratios of methanol-water mixtures and acetonitrile-water mixtures were conducted. In the end, a linear gradient composed of acetonitrile-water was selected because it provided the chromatogram with the best separation while also having minimal background noise.

Meanwhile, for the sample preparation, protein precipitation was chosen over other commonly used methods since it was simpler and cost-effective as well. In this study, methanol was chosen as the precipitating solvent. When added in a 4:1 ratio to the plasma sample, it was able to recover much of the rifampicin in the plasma solution even with a minimal plasma sample (500 uL).

Furthermore, ascorbic acid was added to methanol to reduce the oxidative degradation of rifampicin during sample preparation. Similarly, TFA was also added to the mobile phase such that the acetonitrile and water solvents have

Table 3. Intraday and Interday Precision (%CV) and Accuracy (%error)

Quality Control Sample (µg/mL)	Intraday Run (n=5)			Interday Run (n=25)		
	Mean Concentration ± SD (µg/mL)	%Error	%CV	Mean Concentration ± SD (µg/mL)	%Error	%CV
2 (LLQC)	2.39 ± 0.02	19.56	1.03	2.22 ± 0.18	11.19	8.27
4 (LQC)	4.08 ± 0.21	2.05	5.24	4.13 ± 0.26	3.31	6.23
12 (MQC)	11.85 ± 0.19	-1.26	1.61	11.9 ± 0.37	-0.62	3.08
25 (HQC)	25 ± 0.74	-0.02	2.94	24.56 ± 0.55	-1.76	2.22

%CV – percent coefficient of variation, LLQC – lower limit quality control, LQC – low quality control, MQC – medium quality control, HQC – high quality control

exactly 0.01%TFA. This was done to improve the chromatogram and limit the degradation of rifampicin during analysis.

CONCLUSION

In this study, a UHPLC method for the analysis of rifampicin in human plasma was validated in terms of selectivity, linearity, sensitivity, accuracy, and precision, and the presence of carry-over effect according to the guidelines followed by the Philippine FDA and the ASEAN Guidelines for Harmonisation of Standards. All validation parameters were within the acceptance criteria. The protein precipitation approach in sample cleanup allowed for simple and fast preparation of the sample while also requiring very minimal amounts of plasma. Meanwhile, the chromatographic conditions set allowed for the rapid analysis of the spiked rifampicin samples. The validated method holds great value for drug monitoring of rifampicin-containing drugs. The minimal sample processing using only 500 uL of plasma and the 3-minute UHPLC analysis time make it advantageous when applied to high throughput analyses.

Limitations of the Study

Although the validated UHPLC method for quantification of rifampicin was within the ICH criteria, several limitations must be considered. The method was validated using pooled plasma from a single source, which may not fully account for variability across different individuals or in special populations with specific conditions (e.g., renal or hepatic dysfunction). The use of a single internal standard (rifapentine) may not completely eliminate the matrix effect. Long-term stability was not fully explored in the study which may affect the method's robustness and versatility in diverse patient groups.

Statement of Authorship

All authors certified fulfillment of ICMJE authorship criteria.

Author Disclosure

All authors declared no conflicts of interest.

Funding Source

REFERENCES

1. Flores GP, Alberto IRI, Eala MAB, Cañal JPA. The social determinants of tuberculosis in the Philippines. Lancet Glob Health. 2022 Jan; 10(1):e38. doi: 10.1016/s2214-109x(21)00516-7. PMID:34919852.
2. Agrawal S, Kaur KJ, Singh I, Bhade SR, Kaul CL, Panchagnula R. Determination of rifampicin bioequivalence in a three-drug FDC by WHO and indian protocols: effect of sampling schedule and size. Int J Tuberc Lung Dis. 2005 Jan;9(1):75-80. PMID: 15675554.
3. Singh S, Mariappan TT, Shankar R, Sarda N, Singh B. A critical review of the probable reasons for the poor variable bioavailability of rifampicin from anti-tubercular fixed-dose combination (FDC) products, and the likely solutions to the problem. Int J Pharm. 2001 Oct;228(1-2):5-17. doi: 10.1016/s0378-5173(01)00754-2.
4. Xu J, Jin H, Zhu H, Zheng M, Wang B, Liu C. et al. Oral bioavailability of rifampicin, isoniazid, ethambutol, and pyrazinamide in a 4-drug fixed-dose combination compared with the separate formulations in healthy Chinese male volunteers. Clin Ther. 2013 Feb;35(2): 161-8. doi: 10.1016/j.clinthera.2013.01.003. PMID: 23410999.
5. Luciani-Giacobbe LC, Guzman ML, Manzo RH, Olivera ME. Validation of a simple isocratic HPLC-UV method for rifampicin and isoniazid quantification in human plasma. J Appl Pharm Sci. 2018 Jul;8(7):93-9. doi: 10.7324/japs.2018.8715.
6. Immanuel C, Gurumurthy P, Ramachandran G, Venkatesan P, Chandrasekaran V, Prabhakar R. Bioavailability of rifampicin following concomitant administration of ethambutol or isoniazid or pyrazinamide or a combination of the three drugs. Indian J Med Res. 2003 Sep;118:109-14. PMID: 14700343.
7. Shishoo CJ, Shah SA, Rathod IS, Savale SS, Vora MJ. Impaired bioavailability of rifampicin in presence of isoniazid from fixed dose combination (FDC) formulation. Int J Pharm. 2001 Oct 9;228(1-2): 53-67. doi: 10.1016/s0378-5173(01)00831-6. PMID: 11576768.
8. Ellard GA, Fourie PB. Rifampicin bioavailability: a review of its pharmacology and the chemotherapeutic necessity for ensuring optimal absorption. Int J Tuberc Lung Dis. 1999 Nov;3(11 Suppl 3): S301-8; discussion S317-21. PMID: 10593709.
9. Zhu H, Guo SC, Hao LH, Liu CC, Wang B, Fu L. et al. Relative bioavailability of rifampicin in four Chinese fixed-dose combinations compared with rifampicin in free combinations. Chin Med J (Engl). 2015 Feb 20;128(4):433-7. doi: 10.4103/0366-6999.151061. PMID: 25673441. PMCID: PMC4836242.
10. Hao LH, Guo SC, Liu CC, Zhu H, Wang B, Fu L. et al. Comparative bioavailability of rifampicin and isoniazid in fixed-dose combinations and single-drug formulations. Int J Tuberc Lung Dis. 2014 Dec; 18(12):1505-12. doi: 10.5588/ijtld.13.0647. PMID: 25517820.
11. Temova Rakuša Ž, Roškar R, Klančar Andrejc A, Trdan Lušin T, Faganeli N, Grabnar I, et al. Fast and Simple LC-MS/MS Method for Rifampicin Quantification in Human Plasma. Int J Anal Chem. 2019 Feb 3;2019:4848236. doi: 10.1155/2019/4848236. PMID: 30853987. PMCID: PMC6377990.
12. Chellini PR, Lages EB, Franco PH, Nogueira FH, César IC, Pianetti GA. Development and Validation of an HPLC Method for Simultaneous Determination of Rifampicin, Isoniazid, Pyrazinamide, and Ethambutol Hydrochloride in Pharmaceutical Formulations. J AOAC Int. 2015 Sep-Oct;98(5):1234-9. doi: 10.5740/jaoacint.14-237. PMID: 26525241.
13. Prasanthi B, Ratna JV, Phani RSC. Development and validation of RPHPLC method for simultaneous estimation of rifampicin, isoniazid and pyrazinamide in human plasma. J Anal Chem. 2015 Jul; 70: 1015-22. doi: 10.1134/s1061934815080146.
14. Luciani-Giacobbe LL, Guzman ML, Manzo RH, Olivera ME. Validation of a simple isocratic HPLC-UV method for rifampicin and isoniazid quantification in human plasma. J Appl Pharm Sci. 2018 Jul; 8(07): 93-9. doi: 10.7324/JAPS.2016.60702.
15. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH): ICH guideline M10 on bioanalytical method validation and study sample analysis Geneva, Switzerland. 2022.
16. Goutal S, Auvity S, Legrand T, Hauquier F, Cisternino S, Chapy H, et al. Validation of a simple HPLC-UV method for rifampicin determination in plasma: Application to the study of rifampicin arteriovenous concentration gradient. J Pharm Biomed Anal. 2016 May 10;123:173-8. doi: 10.1016/j.jpba.2016.02.013. PMID: 26907700.
17. Nahar L, Onder A, Sarker SD. A review on the recent advances in HPLC, UHPLC and UPLC analyses of naturally occurring cannabinoids (2010-2019). Phytochem Anal. 2020 Jul;31(4):413-457. doi: 10.1002/pca.2906. PMID: 31849137.
18. Cielecka-Piontek J, Zalewski P, Jelińska A, Garbacki P. UHPLC: the greening face of liquid chromatography. Chromatographia. 2013: 76:1429-37. doi: 10.1007/210337-013-2434-6.