Journal of Research in Pharmacy

Research Article

www.jrespharm.com

Therapeutic Drug Monitoring in Pediatric Patients Treated with Anti-Tuberculosis Medications by High Performance Liquid Chromatography

Betul OKUYAN[1,a], Fatih TOK[2,a], Sevgi KARAKUŞ[2], Nazan DALGIÇ[3], Erkan ÇAKIR[4], Levent MIDYAT[5], Bedia KOÇYİĞİT-KAYMAKÇIOĞLU[2], Ufuk Eren BERK[4], Fikret Vehbi İZZETTIN[6], Sevim ROLLAS[2], Mesut SANCAR[1,*]

[a]The authors equally contributed to this study

[1] Department of Clinical Pharmacy, Faculty of Pharmacy, Marmara University, Başıbüyük 34854, Istanbul, Türkiye.
[2] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Marmara University, Başıbüyük 34854, Istanbul, Türkiye.
[3]Division of Pediatric Infectious Diseases, University of Health Sciences, Şişli Hamidiye Etfal Training and Research Hospital, Sarıyer 34453, Istanbul, Türkiye.
[4]Department of Pediatrics, Faculty of Medicine, Bezmialem Vakif University, Fatih 34093, Istanbul, Türkiye.
[5]Division of Pediatric Pulmonology, Sureyyapasa Chest Diseases and Thoracic Surgery Teaching Hospital, Maltepe 34844, Istanbul, Türkiye.
[6]Department of Clinical Pharmacy, Faculty of Pharmacy, Bezmialem Vakif University, Fatih 34093, Istanbul, Türkiye.

*Corresponding Author. E-mail: sancarmesut@yahoo.com (M.S.); Tel. +90216 777 5200.

Received: 15 July 2022 / Revised: 03 September 2022 / Accepted: 05 September 2022

ABSTRACT: The aim of this study is to perform therapeutic drug monitoring for isoniazid (INH), rifampicin (RIF), and pyrazinamide (PZA) in pediatric tuberculosis patients. The study was carried out in 3 different training-research hospitals in Istanbul, Türkiye between 2011 and 2012. The pediatric patients (aged ≤14 years) who initiated the standard primary anti-tuberculosis therapy were included in this study. The serum samples were collected 3 hours after the first medication doses were given on the 5th day of treatment. Chromatographic experiments were performed on an Agilent 1100 High-Performance Liquid Chromatography (HPLC) system, and the separation was carried out on a Nova-Pak C_{18} (3.9x150 mm, 5 µm, Merck) analytical column. In this HPLC method, the gradient elusion delivered 3% to 40% (v/v) acetonitrile in phosphate buffer was used, and diode array detector. Twenty-three children (60.9% male) patients were included with a mean age of 111.70 ± 59.94 months. Plasma levels were measured sub-therapeutically for INH in 14, RIF in 10, and PZA in 5 patients, according to the normal range of adult patients. Maximum plasma concentrations after three hours were found between 0.53-14.02 mg/L for INH, 11.17-60.39 mg/L for PZA, 2.15-16.75 mg/L for RIF. In conclusion, this method has been successfully applied to simultaneously determine RIF, INH, and PZA plasma levels in pediatric tuberculosis patients. RIF and INH plasma levels were found to be lower in pediatric patients with tuberculosis compared to target range of adult patients.

KEYWORDS: HPLC; tuberculosis; pediatric patients; therapeutic drug monitoring.

1. INTRODUCTION

Tuberculosis (TB) is one of the most causes of death globally [1, 2]. Tuberculosis has been declared a global emergency by the World Health Organization (WHO) since 1993 [3]. It was estimated that there were nearly 10 million new cases and 1.2 million deaths in 2019 [4]. Tuberculosis was a much more severe problem in the past; today, there are many alternatives for treatment [5]. Medications such as isoniazid (INH), pyrazinamide (PZA), ethambutol (EMB), and rifampicin (RIF) are recommended for the first-line treatment of tuberculosis [6, 7]. The therapy regimen for TB patients is recommended with these first-line antituberculosis medications for the first two months; then, the regimen is continued for the next four

How to cite this article: Okuyan B, Tok F, Karakuş S, Dalgıç N, Çakır E, Midyat L, Koçyiğit-Kaymakçıoğlu B, Berk UE, İzzettin FV, Rollas S, Sancar M. Therapeutic Drug Monitoring In Pediatric Patients Treated with Anti-Tuberculosis Medications by High Performance Liquid Chromatography. J Res Pharm. 2022; 26(6): 1907-1914.

ISSN: 2630-6344

http://dx.doi.org/10.29228/jrp.281

months with isoniazid and rifampicin [8]. Although these regimens are quite efficient for most medication-susceptible TB patients, some patients experience some difficulties such as poor response, drug-drug interactions, adverse effects, and bacterial resistance [9, 10].

Therapeutic drug monitoring (TDM) is the measurement of serum/plasma concentrations of medications to determine each patient's therapeutic dosage [11, 12]. TDM is used primarily in patients at high risk of treatment failure or delayed response to improve treatment response in individual TB patients [13].

The use of TDM in TB patients has many benefits, such as evaluating potential drug-drug interactions, determining concordance to therapy, preventing antimicrobial resistance, and avoiding toxicity [14]. By monitoring medication levels, the aim is to provide maximum therapeutic benefit and minimum toxic effect in treatment [15]. Although it is widely recognized that children's pharmacokinetics differ significantly from adults', pharmacokinetic studies in children are often not performed. Therefore, there is a need for TDM of anti-TB medications in children [16]. The most common advantages of TDM in pediatric patients were: evaluation of treatment effectiveness, avoiding potential adverse events, and providing medication adherence [17].

To analyze medications in different biological samples are required an efficient separation technique and a sensitive detection method. High-performance liquid chromatography (HPLC) is one of the most efficient and robust separation techniques due to its convenience, simple operation, strong separation ability, and wide sample application [18]. The main goal of this study was to develop a method for determining the plasma concentration of INH, RIF, and PZA in pediatric patients with tuberculosis by using the RP-HPLC method developed previously by Unsalan et al [19]. The secondary aim of the study was to classify the plasma concentration of the anti-tuberculosis medications according to the normal range.

2. RESULTS

Twenty-three pediatric patients receiving primary tuberculosis treatment were included in this study. The mean age of the children was 111.70 ± 59.94 months (min-max: 6-192 months), and the mean body weight was 32.00 ± 13.65 kg Fourteen (60.9%) were male. Of them, 30.4% had any Bacille Calmette-Guérin (BCG) vaccination scar. While 18 patients were diagnosed with pulmonary tuberculosis, 5 patients were treated for extra-pulmonary tuberculosis.

Acetonitrile in phosphate buffer was used as the mobile phase. All medications (INH, PZA and, RIF) and internal standard (acetanilide) resolved satisfactorily in the mobile phase, and their signals were determined separately without any masking interfering peaks from medication-free plasma. The analytical run was completed with gradient elusion at 20 min because of this good separation. The retention time of INH, PZA, and RIF were detected at 3.8, 4.6 and, 10.2 minutes, respectively. All signals were recorded by a diode array detector at the different wavelengths: 215, 248, 261 and, 475 nm. However, the optimum wavelength was found, especially 261 nm, to determine PZA, RIF, and INH. It was given the chromatograms of blank plasma and plasma samples of the patient receiving PZA, RIF, and INH in Figure 1.

Journal of Research in Pharmacy
Research Article

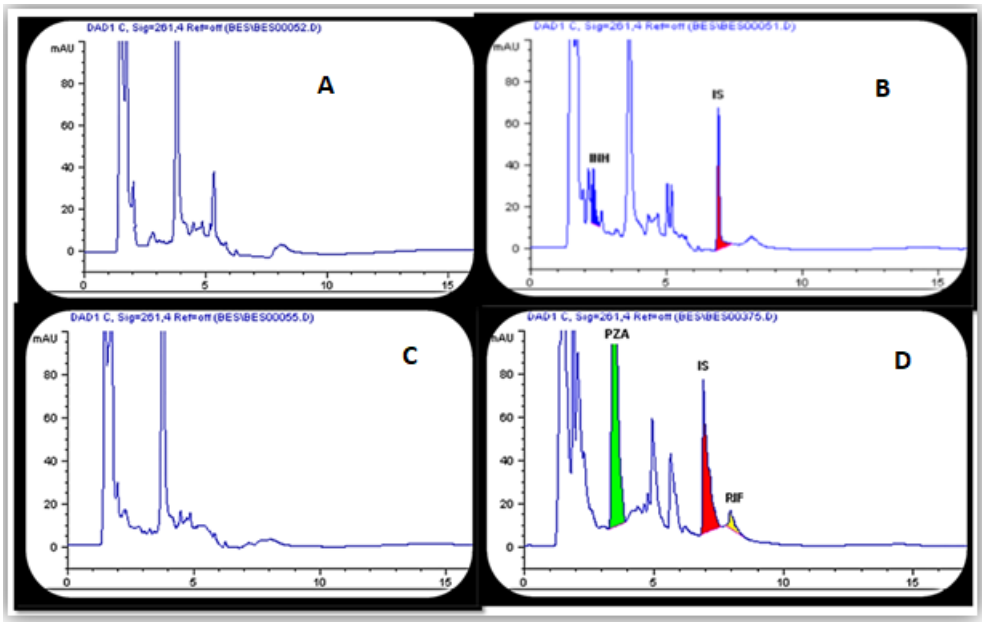

Figure 1. Chromatograms of blank plasma (A, C) and plasma samples of the patient receiving INH (B) and PZA, RIF (D).

Isoniazid, pyrazinamide, and rifampicin blood levels of 23 pediatric patients receiving primary tuberculosis treatment are given in Table 1. The percentage of patients outside the therapeutic range based on the adult reference range is shown in Table 2. Comparative analyzes according to sociodemographic characteristics such as age and gender and tuberculosis type are presented in Table 3.

Table 1. Therapeutic concentration of INH, RIF, and PZA in pediatric patients (n=23)

Medications	**Median Cmax (IQR) at 3h mg/dL**	**Range (Min-Max) for Cmax, mg/dL**	**Adults' normal range mg/dL**[20]
INH	2.41 (1.88-7.42)	0.53-14.02	3-6
RIF*	4.60 (2.61-9.44)	2.15-16.75	8-14
PZA*	32.12 (14.70-49.74)	11.17-60.39	20-50

Cmax: The maximum plasma concentration, IQR: Inter quartile ratio
*Values <LOQ (Limit of Quantitation) were not included in the analysis.

Table 2. The classification of pediatric patients' plasma concentrations of anti-tuberculosis medications according to the adults'normal range (n=23)

Medications	**Number of patients with sub-therapeutic plasma levels n (%)**	**Number of patients with supra-therapeutic plasma levels n (%)**
INH	14 (60.9)	4 (17.4)
RIF	10 (43.5)	2 (8.7)
PZA	5 (21.7)	2 (8.7)

http://dx.doi.org/10.29228/jrp.281
J Res Pharm 2022; 26(6): 1907-1914

Table 3. Comparison of plasma concentrations of anti-tuberculosis medications according to the patients' sociodemographic characteristics (n=23)

Characteristics		Median Cmax (IQR) at 3h mg/dL		
		INH	RIF	PZA
Gender	Female	3.98 (1.83-10.86)	3.59 (2.28-13.71)	35.85 (16.14-49.59)
	Male	2.38 (1.62-6.30)	6.19 (3.19-9.44)	31.22 (14.38-51.26)
	p	NS	NS	NS
Age	12 years and over	3.43 (2.29-10.37)	5.39 (2.91-10.34)	33.98 (14.38-56.69)
	< 12 years	1.60 (0.60-5.05)	2.63 (1.98-9.42)	22.28 (13.421-39.83)
	p	0.048*	NS	NS
Type of Tuberculosis	Pulmonary	2.38 (1.50-7.75)	4.60 (2.64-11.25)	24.43 (13.45-44.97)
	Extra-pulmonary	3.98 (2.41-7.57)	4.90 (1.88-14.47)	47.32 (36.58-59.25)
	p	NS	NS	NS

*p<0.05, statistical significance, NS: Non-significant

The validation parameters of linearity, system appropriateness, accuracy, and recovery were tested by using the previous method. The correlation coefficients have been calculated in a concentration range of 0.9–15 mg/L for INH (r=0.9975), 8–80 mg/L for PZA (r=0.9984), 4.5–45 mg/L for RIF (r=0.9983). The percentages of relative recovery were in the range of 91.5–111.2 of nominal values of INH, PZA, and RIF. The relative standard deviations (RSD%) for three different concentrations of INH, PZA, and RIF in plasma in the same-day study varied 1.6–3.93 mg/dL, 2.85–3.37 mg/dL, and 2.86–11.07 mg/dL, respectively, whereas those in the day-to-day study varied 1.67–3.34 mg/dL, 2.71–10.22 mg/dL, and 2.24–7.13 mg/dL respectively. The lower limits of quantification were 0.6 mg/L for INH, 1.5 mg/L for PZA, and 0.7 mg/L for RIF [19]. In conclusion, the method we applied in pediatric patients was sensitive and reliable for routine TDM of INH, PZA, and RIF.

3. DISCUSSION

A previous study in tuberculosis patients showed that the method for monitoring INH, PZA, and RIF using HPLC is valid and reliable. It was demonstrated that this method could be applied in the daily practice of TDM in the poor response of tuberculosis patients. In our previous study, blood samples taken from 25 adult patients were studied, and plasma concentrations of INH, PZA, and RIF were measured using the developed and validated method. Therefore the INH, RIF, and PZA peaks showed no interference from the endogenous components. As a result of the previous study, plasma concentration ranges were determined as 0.98-6.27 mg/L for INH, 11.05-47.26 mg/L for PZA, and 5.09-33.20 mg/L for RIF [19]. We applied the same method to blood samples taken for pediatric patients in this study. This method, which our research group developed, was successfully applied in this study.

Antituberculosis medications in children have a wide pharmacokinetic variability and may have serum concentrations below the therapeutic level because of their continuous growth and development. A study in Africa found that the new WHO-recommended doses of rifampicin and pyrazinamide did not reach adequate serum concentrations in children under 12 kg. In particular, serum rifampicin levels were lower in children than adults, regardless of weight [21, 22]. Another study carried out in India assessed the serum concentrations achieved with isoniazid (10 mg/kg) and pyrazinamide (35 mg/kg) and compared them to nutritional status and age. The Cmax of INH in children <3 years was 3.18 µg/mL, and for children,>3 years was 3.05 µg/mL. The Cmax of PZA for children <3 years was 29.22 µg/mL, and for >3 years was 37.12 µg/mL. While the pyrazinamide concentration was lower in children under the age of three, isoniazid did not show a significant difference when theis variable was evaluated. In addition to, higher doses of INH in the revised WHO protocols have demonstrated adequate medication levels, particularly in younger children. However, increasing the dose of PZA did not significantly increase the Cmax values in children <3 years

[23]. In our study, no difference was observed between the plasma levels of drugs in terms of gender and tuberculosis type. Consistent with the literature, although plasma drug levels were lower in patients younger than 12 years of age compared to children aged 12 years and older, this difference was only significant in terms of INH levels.

Kumar et al. found pyrazinamide concentrations in 22 children, 45% below the therapeutic range, 32% within the therapeutic range, and 23% above the therapeutic range. The range values of pyrazinamide were found variations [24]. In our study, PZA levels in 23 pediatric patients were below the therapeutic range in approximately 22%, within the therapeutic range in about 70%, and above the therapeutic range in around 9%. Although PZA levels in our study were different from those in the Kumar study, the number of patients with subtherapeutic levels of RIF and INH was high.

WHO revised dosing guidelines for children under 12 years of age in 2010 after studies showed lower medication exposure in children than adults treated with the same mg/kg doses, Doses of rifampin were increased by 50% to 15 (10-20) mg/kg, for isoniazid to 10 (7-15) mg/kg by 100%, and 35 (30-40) mg/kg for pyrazinamide. Recent studies evaluating these doses show that although higher pediatric weight bands achieve similar mean rifampin and pyrazinamide exposure to adults, children under 12 kg remain below that in adults [22, 25, 26]. According to the results of these studies and our results demonstrated that the maximum plasma concentrations of INH and RIF in children have been reported to be low and variable. Based on the results of our study in line with the studies in the literature and the recommendations of the WHO, we think that drug doses should be reconsidered in pediatric tuberculosis patients.

There are some barriers to TDM in children with tuberculosis, and therefore it has not become widely applicable. Some of these obstacles are; the lack of a definite blood level in children and the fact that it is usually compared with adult levels, parents' reluctance to collect blood from children, laboratory conditions, and high cost [27]. However, since we are faced with the fact that drug levels may be low in children with tuberculosis despite dose revisions, we think that TDM will be beneficial, at least in children who do not respond adequately to treatment.

This study has some limitations: Although the study was multicenter, the number of patients was relatively low. Therefore, the relationship between low plasma drug concentrations and some clinical conditions could not be analyzed. It is not possible for these results to reflect all children with tuberculosis.

4. CONCLUSION

This study highlights the appropriateness of the HPLC method for monitoring antituberculosis medications in pediatric patients with tuberculosis. In addition, therapeutic plasma levels of anti-tuberculosis medications were lower and more variable in pediatric patients than in adult patients. The findings of this study contributed to the crucial role of TDM in pediatric patients receiving anti-tuberculosis treatment for individual management of tuberculosis.

5. MATERIALS AND METHODS

5.1. Chemicals

Active ingredients INH, PZA, and RIF were obtained from KOÇAK FARMA (Istanbul, Turkey). Acetanilide, potassium dihydrogen phosphate and trichloroacetic acid (TCA) were obtained from Sigma-Aldrich (St. Louis, MO). Acetonitrile and methanol were HPLC grade and supplied from Merck (Darmstadt, Germany). HPLC grade water was prepared from a Milli-Q RG system (Molsheim, France).

5.2. Apparatus and Chromatographic Condition

HPLC analysis was carried out using the Agilent 1100 Series (Waldbronn, Germany). The parameters of the study were determined as 3.9x150 mm Nova-Pak C18 column with 5 µm particle size, diodearray detector at four different wavelengths (215, 248, 261, and 475 nm), mobile phase as potassium dihydrogen phosphate, and acetonitrile, and flow rate of 0.8 ml/min at 25 °C. Gradient elution was accomplished using 10 mmol potassium dihydrogen phosphate (pH 6.24) as solvent A and acetonitrile as solvent B for better separation. The gradient elution system of 0–1 min, 0–3% B; 1–5.5 min, 3–40% B; 5.5–12 min, 40–3% B; 12–20 min, 3–3% B was designated. The latter composition, at least 8 min, was maintained. Chromatographic data were recorded using Agilent Chemstation software.

5.3. Preparation of Standard Solutions

INH, PZA, and RIF stock solutions were prepared for 1 g medication/1L water concentration. The calibration concentration ranges for INH, PZA, and RIF were chosen considering the expected steady-state concentration levels in plasma. Standard solutions were prepared for calibration by diluting the stock solutions with blank plasma to final concentrations of 4.5-45 mg/L for RIF, 8-80 mg/L for PZA, and 0.9-15 mg/L for INH. Acetanilide (1g) was dissolved in acetonitrile (1 L) as an internal standard. Then the stock solution of acetanilide was diluted with acetonitrile to a concentration of 6.25 mg/L and diluted with 10% TCA to a concentration of 25 mg/L. The calibration graphs were obtained by plotting the peak area of medications to the internal standard against the known concentrations of INH, PZA, and RIF added to the medication-free plasma.

5.4. Sample Collection

The study was carried out in 3 different training-research hospitals in Istanbul, Türkiye between April 2011 and April 2012. The pediatric patient (aged ≤14 years) with tuberculosis (including pulmonary and/or extrapulmonary tuberculosis), who initiated the standard primary anti-tuberculosis therapy (10-15 mg/kg of RIF, 10-15 mg/kg of INH, 20-40 mg/kg of PZA and 15-25 mg/kg of EMB) were included to this study. Patients who were Human Immunodeficiency Virus (HIV) positive had known immunodeficiency and chronic disease had a history of gastrointestinal disease or diarrhea, had kidney and liver failure, and had malnutrition were excluded from the study. Written informed consent was taken from their parents. Demographic data (including age and sex) was collected. The serum samples were collected 3 hours after the first medication doses were given on the 5th day of treatment and kept at -20°C.

5.5. Sample Preparation

For PZA and RIF, a 100 µL plasma sample was mixed using a Vortex mixer and ultrasound with 200 µL of a solution of acetanilide 6.25 mg/L in acetonitrile. Then, the mixture was centrifuged. The supernatant (150 µL) was evaporated at room temperature for two days. The residue was reconstituted in 30 µL water and injected into the column. For INH, a 100 µL plasma sample was mixed with 50 µL 10% TCA containing 25 mg/L acetanilide in acetonitrile. Then, the mixture was centrifuged. The supernatant (50 µL) was neutralized with ammonium acetate (0.5 M, pH 8.4) and directly injected into the column.

Ethical Approval

This study was approved by Marmara University Faculty of Medicine Research Ethics Committee with decision number 47 on March 3, 2011.

Acknowledgements: This study was supported by Marmara University Scientific Research Projects Unit with the project number SAG-A-130511-0128.

Author contributions: Concept – M.S., B.O., N.D.; Design – B.O., N.D., S.K. B.K, M.S.; Supervision – S.R., FV.İ.; Resources – M.S.; Materials – M.S., N.D., E.Ç., L.M., UE.B.; Data Collection and/or Processing – B.O., F.T., S.K., N.D., E.Ç., L.M., UE.B.. M.S.; Analysis and/or Interpretation – B.O., F.T., S.K., B.K., M.S.; Literature Search – F.T., M.S.; Writing – B.O., F.T., M.S.; Critical Reviews – B.O., F.T., S.K., N.D., E.Ç., L.M., B.K., UE.B., S.R., FV.İ., M.S.

Conflict of interest statement: The authors declared no conflict of interest in the manuscript.

REFERENCES

[1] Pitucha M, Karczmarzyk Z, Swatko-Ossor M, Wysocki W, Wos M, Chudzik K, Ginalska G, Fruzinski A. Synthesis, in vitro screening and docking studies of new thiosemicarbazide derivatives as antitubercular agents. Molecules. 2019;24(2):251.[CrossRef]

[2] Nguyen PC, Delorme V, Bénarouche A, Guy A, Landry V, Audebert S, Pophillat M, Camoin L, Crauste C, Galano JM, Durand T, Brodin P, Canaan S, Cavalier JF. Oxadiazolone derivatives, new promising multi-target inhibitors against M. tuberculosis. Bioorg Chem. 2018;81:414-424.[CrossRef]

[3] Castelo-Branco FS, de Lima EC, Domingos JLO, Pinto AC, Lourenço MCS, Gomes KM, Costa-Lima MM, Araujo-Lima CF, Aiub CAF, Felzenszwalb I, Costa TEMM, Penido C, Henriques MG, Boechat N. New hydrazides derivatives of isoniazid against Mycobacterium tuberculosis: Higher potency and lower hepatocytotoxicity. Eur J Med Chem. 2018;146:529-540. [CrossRef]

[4] Global tuberculosis report 2020. Geneva: World Health Organization; 2020. Licence: CC BY-NC-SA 3.0 IGO.

[5] Igarashi M. Development of new antituberculosis drugs from natural products. Biosci Biotechnol Biochem. 2017;81(1):32-37. [CrossRef]

[6] Halicki PCB, Ferreira LA, De Moura KCG, Carneiro PF, Del Rio KP, Carvalho TDSC, Pinto MCFR, Silva PEA, Ramos DF. Naphthoquinone derivatives as scaffold to develop new drugs for tuberculosis treatment. Front Microbiol. 2018;9:673.[CrossRef]

[7] Ugwu DI, Ezema BE, Eze FU, Ugwuja DI. Synthesis and structural activity relationship study of antitubercular carboxamides. Int J Med Chem. 2014; 2014:614808.[CrossRef]

[8] Zheng Y, Xu N, Hu X, Zhang Q, Liu Y, Zhao Q. Development and application of a LC-MS/MS method for simultaneous quantification of four first-line antituberculosis drugs in human serum. J Anal Methods Chem. 2020;2020:8838219. [CrossRef]

[9] Oliveira JRS, Shiguemoto CYK, Neves AR, Moreira FMF, Gomes GB, Perdomo RT, Barbosa SL, Guerrero PG, Croda J, Baroni ACM. Design, synthesis and antitubercular activity of novel isoniazid−cyclic−amine−azachalcones hybrids. J Braz Chem Soc. 2020;31(6):1284-1295.[CrossRef]

[10] Martinez R, Zamudio GJN, Pretelin-Castillo G, Torres-Ochoa RO, Medina-Franco JL, Pinzon CIE, Miranda MS, Hernandez E, Alanis-Garza B. Synthesis and antitubercular activity of new *N*-[5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl]-(nitroheteroaryl)carboxamides. Heterocycl. Commun. 2019;25:52–59.[CrossRef]

[11] Johannessen Landmark C, Johannessen SI, Patsalos PN. Therapeutic drug monitoring of antiepileptic drugs: current status and future prospects. Expert Opin Drug Metab Toxicol. 2020;16(3):227-238. [CrossRef]

[12] Martial LC, Kerkhoff J, Martinez N, Rodríguez M, Coronel R, Molinas G, Roman M, Gomez R, Aguirre S, Jongedijk E, Huisman J, Touw DJ, Perez D, Chaparro G, Gonzalez F, Aarnoutsa RE, Alffenaar JW, Magis-Escurra C. Evaluation of dried blood spot sampling for pharmacokinetic research and therapeutic drug monitoring of anti-tuberculosis drugs in children. Int J Antimicrob Agents. 2018;52(1):109-113.[CrossRef]

[13] Saktiawati AMI, Harkema M, Setyawan A, Subronto YW, Sumardi, Stienstra Y, Aarnoutsa RE, Magis-Escurra C, Kosterink JGW, Werf TS, Alffenaar JWC, Sturkenboom MGG. Optimal sampling strategies for therapeutic drug monitoring of first-line tuberculosis drugs in patients with tuberculosis. Clin Pharmacokinet. 2019;58(11):1445-1454.[CrossRef]

[14] Märtson AG, Burch G, Ghimire S, Alffenaar JWC, Peloquin CA. Therapeutic drug monitoring in patients with tuberculosis and concurrent medical problems. Expert Opin Drug Metab Toxicol. 2021;17(1):23-39.[CrossRef]

[15] Babalik A, Mannix S, Francis D, Menzies D. Therapeutic drug monitoring in the treatment of active tuberculosis. Can Respir J. 2011;18(4):225-229.[CrossRef]

[16] Zuur MA, Bolhuis MS, Anthony R, den Hertog A, van der Laan T, Wilffert B, Lange W, Soolingen D, Alffenaar JWC. Current status and opportunities for therapeutic drug monitoring in the treatment of tuberculosis. Expert Opin Drug Metab Toxicol. 2016;12(5):509-521.[CrossRef]

[17] Seddon JA, Furin JJ, Gale M, Del Castillo Barrientos H, Hurtado RM, Amanullah F, Ford N, Starke JR, Schaaf HS, Sentinel Project on Pediatric Drug-Resistant Tuberculosis. Caring for children with drug-resistant tuberculosis: practice-based recommendations. Am J Respir Crit Care Med. 2012;186(10):953-964. [CrossRef]

[18] Wróblewski K, Petruczynik A, Tuzimski T, Prajsnar K, Przygodzka D, Buszewicz G, Karakula-Juchnowicz H, Rog J, Morylowska-Topolska J, Waksmundzka-Hajnos M. Optimization of chromatographic systems for analysis of selected psychotropic drugs and their metabolites in serum and saliva by HPLC in order to monitor therapeutic drugs. Open Chem. 2019;17:1361-1373.[CrossRef]

[19] Unsalan S, Sancar M, Bekce B, Clark PM, Karagoz T, Izzettin FV, Rollas S. Therapeutic monitoring of isoniazid, pyrazinamide and rifampicin in tuberculosis patients using LC. Chromatographia. 2005;61:595-598.[CrossRef]

[20] Verhagen LM, Lopez D, Hermans PWM, Warris A, Groot R, Garcia JF, Waard JH, Aarnoutse RE. Pharmacokinetics of anti-tuberculosis drugs in Venezuelan children younger than 16 years of age: supportive evidence for the implementation of revised WHO dosing recommendations. Trop Med Int Health. 2012;17(12):1449-1456.[CrossRef]

[21] Tahan TT, Gabardo BMA, Rossoni AMO. Tuberculosis in childhood and adolescence: a view from different perspectives. J Pediatr (Rio J). 2020;96(1):99-110. [CrossRef]

[22] McIlleron H, Chirehwa MT. Current research toward optimizing dosing of first-line antituberculosis treatment. Expert Rev Anti Infect Ther. 2019;17(1):27-38. [CrossRef]

[23] Dayal R, Singh Y, Agarwal D, Kumar M, Swaminathan S, Ramachandran G, Kumar S, Narayan S, Goyal A, Kumar AKH. Pharmacokinetic study of isoniazid and pyrazinamide in children: impact of age and nutritional status. Arch Dis Child. 2018;103(12):1150-1154.[CrossRef]

[24] Hemanth Kumar AK, Kumar A, Kannan T, Bhatia R, Agarwal D, Kumar S, Dayal R, Singh SP, Ramachandran G. Pharmacokinetics of second-line antituberculosis drugs in children with multidrug-resistant tuberculosis in India. Antimicrob Agents Chemother. 2018;62(5): 02410-02417.[CrossRef]

[25] Horita Y, Alsultan A, Kwara A, Antwi S, Enimil A, Ortsin A, Dompreh A, Yang H, Wiesner L, Peloquin CA. Evaluation of the adequacy of WHO revised dosages of the first-line antituberculosis drugs in children with tuberculosis using population pharmacokinetic modeling and simulations. Antimicrob Agents Chemother. 2018;62(9):00008-00018.[CrossRef]

[26] Bekker A, Schaaf HS, Draper HR, van der Laan L, Murray S, Wiesner L, Donald PR, McIIIeron HM, Hesseling AC. Pharmacokinetics of rifampin, isoniazid, pyrazinamide, and ethambutol in infants dosed according to revised WHO-recommended treatment guidelines. Antimicrob Agents Chemother. 2016;60(4):2171-2179. [CrossRef]

[27] Cruz AT, Starke JR. Monitoring treatment of childhood tuberculosis and the role of therapeutic drug monitoring. Indian J Pediatr. 2019;86(8):732-739. [CrossRef]