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Original Article

Biochemistry Section

High-performance Liquid Chromatography based Methods for Determination of Antitubercular Drugs in Urine Samples: A Scoping Review

SHILPA MANISH UPADHYAY[1], ARCHANA DHOK[2], ZAHIRUDDIN SYED QUAZI[3]

ABSTRACT

Introduction: Tuberculosis (TB) is a contagious disease and is one of the leading causes of death worldwide. TB is primarily caused by Mycobacterium Tuberculosis (MTB) and the percentage of Multi-Drug Resistance TB (MDR-TB) and Extensively Drug Resistance TB (XDR-TB) is increasing daily in many developing countries.

Aim: To summarise different High-performance Liquid Chromatography (HPLC)-based methods for checking treatment adherence and drug monitoring of patients by estimating the amount of Anti-tubercular (Anti-TB) drugs in urine samples.

Materials and Methods: The authors conducted a search and screened various databases (PubMed, Scopus, Web of Science, Google Scholar) using different keywords between April 2023 and June 2023. The authors included original research articles, clinical trials, and observational studies that focused on estimating anti-TB drugs in urine samples using HPLC. The authors excluded articles that employed methods other than HPLC for drug estimation in urine samples. A total of 13 articles were included in this review.

Results: The authors identified 296 articles from different electronic databases and four articles from other sources (Google Scholar, ResearchGate, etc.). Ultimately, 13 articles were included describing HPLC-based methods for determining anti-TB drugs in urine samples. Data was extracted focusing on mobile phases and sample preparation/extraction procedures. Two articles of 2004 and 2014 reported simple mobile phases and sample preparation methods for estimating rifampicin and isoniazid. Additionally, articles published in the last five years have employed simple mobile phases with minimal or no extraction procedures.

Conclusion: The present review summarises various HPLC-based methods reported in the literature, as it is considered the gold standard method for checking treatment adherence in TB patients. Urine samples were chosen for ease of collection, particularly from patients of different age groups, including the paediatric population. This review highlights the need for more HPLC-based methods with simple mobile phases and extraction procedures for early detection of anti-TB drugs in resource-poor settings.

Keywords: Adherence, Contagious disease, Multi-drug resistance, Tuberculosis

INTRODUCTION

The TB is a contagious disease and one of the prominent causes of death worldwide [1]. It is also a significant reason for ill-health caused by MTB [1]. According to the World Health Organisation (WHO), approximately 1.6 million people globally lost their lives to TB in 2021 [2]. The majority of new TB cases are reported in the WHO South-East Asian Region (WHO-SEAR) (46%), Africa (23%), and the Western Pacific (18%) [2]. TB is highly prevalent in developing countries, many of which are burdened with a high TB incidence. India is also among the countries with a high TB burden [3]. Rapid increases in TB cases in these countries are attributed to poor diagnosis, non adherence to TB treatment, and lack of awareness about TB, among other factors.

For the treatment of TB, WHO has recommended a standardised regimen consisting of two phases [4]. In the 1950s, anti-TB drugs were discovered, and during the 1960s and 1970s, the illness was believed to be entirely curable and manageable [5]. However, TB cases began to rise again in the 1980s due to the emergence of immunocompromised conditions such as HIV and drug-resistant strains of TB bacteria resulting from mutations. The primary cause of drug-resistant TB was the patient's failure to comply with treatment [5]. MDR-TB remains a public health crisis and a threat to health security [2]. In high TB-burden countries like India, there has been an uncontrolled increase in cases of MDR-TB and XDR-TB. This situation arises when people with drug-resistant TB do not have access to treatment, anti-TB drugs are misused through false prescriptions by healthcare providers, patients prematurely stop treatment, or poor-quality drugs are used [3]. Both MDR-TB and XDR-TB pose an increasing threat to the success of anti-TB programs. The development and adoption of new methods are urgently required to be implemented quickly in hospitals or clinical laboratories as a standard analytical tool for monitoring treatment adherence in patients undergoing Directly Observed Short Course (DOTS) therapy and adjusting their future therapeutic doses [6].

According to the WHO Global Tuberculosis Report 2020, the most recent challenges in managing TB include ensuring equal access to timely and high-quality diagnosis, treatment, prevention, and care [7]. However, poor treatment outcomes and ineffective TB control worldwide are associated with non compliance with the TB treatment regimen [8].

Therefore, in order to achieve the end TB milestone by 2030, there is a need to take proper steps and measures to increase patients' awareness about TB for early diagnosis and adherence to the treatment regimen, especially in developing countries. This review focuses on HPLC-based methods for determining anti-tubercular drugs in urine samples. HPLC is a column-based separation method that uses ion exchange, adsorption, and partition to identify and separate different compounds. It is considered the gold standard method [9]. Many routine analytical and bioanalytical methods are practiced to check patient adherence to drug therapy in biological

fluids such as blood, saliva, serum, and urine [9]. Urine samples were chosen for the ease of collecting samples from patients of different age groups, including the paediatric population.

The present review summarises HPLC-based simple, rapid, sensitive, and cost-effective methods to monitor anti-tubercular drug adherence in a TB patient's urine sample that can be easily applicable in low-resource settings.

MATERIALS AND METHODS

Criteria for considering articles: The authors searched different databases such as PubMed, Scopus, Web of Science, and Google Scholar and included articles on HPLC-based methods for estimating drugs in human urine using relevant keywords. Full journal publications were required for inclusion. The authors excluded articles that used other methods for drug estimation in urine samples, such as colorimetry, mass spectrometry, thin-layer chromatography, etc. The authors did not include articles that used biological fluids other than urine, such as plasma, blood, cerebrospinal fluid, etc. However, the included articles that estimated drugs in urine and other biological fluids, such as plasma, and authors only extracted data for urine samples from those articles. Articles were included from the last 25 years, i.e., from 1998 to 2023, and from all demographic areas. Articles published before 1998 were excluded. The search was restricted to articles published in English only and excluded those published in other languages such as Korean, Japanese, etc. The style of Cochrane reviews were followed in writing this article to maintain a high-quality review. The authors conducted the search between April and June 2023 and presented search strategies for PubMed, Scopus, and Web of Science here:

Search strategy for PubMed:

(((#1) AND (#2)) AND (#3)) AND (#4)
((((urine[Title/Abstract]) OR (blood[Title/Abstract])) OR (plasma[Title/Abstract])) OR ("biological fluids"[Title/Abstract])) OR ("fluids and secretions/analysis"[MeSH Terms])
(((((“anti-tubercul*”[Title/Abstract]) OR (antitubercul*[Title/Abstract])) OR (“anti-TB”[Title/Abstract])) OR (“TB drug*”[Title/Abstract])) OR (“TB regimen”[Title/Abstract])) OR (“antibiotics, anti-tubercular/analysis”[MeSH Terms])
(((((((HPLC[Title/Abstract]) OR (“liquid chromatography”[Title/Abstract])) OR (LC[Title/Abstract])) OR (“analytical method*”[Title/Abstract])) OR (Validation [Title/Abstract])) OR (estimation[Title/Abstract])) OR (“high performance liquid chromatography”[Title/Abstract])) OR (“chromatography, high pressure liquid/methods”[MeSH Terms])
(((tubercul*[Title/Abstract]) OR (“mycobacterium tubercul*”[Title/Abstract])) OR (TB[Title/Abstract])) OR (“mycobacterium tuberculosis”[MeSH Terms])

Search strategy for Scopus:

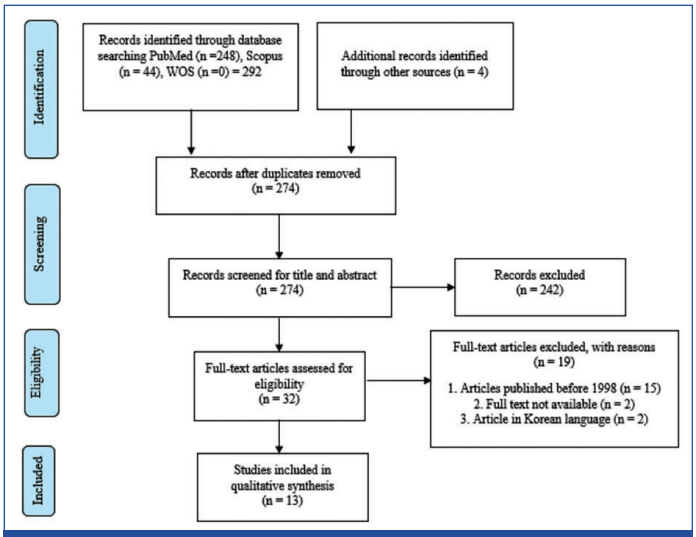
TITLE-ABS-KEY (tubercul*) OR TITLE-ABS-KEY (“mycobacterium tubercul*”) OR TITLE-ABS-KEY (TB) AND TITLE-ABS-KEY (HPLC) OR TITLE-ABS-KEY (“high performance liquid chromatography”) OR TITLE-ABS-KEY (analytical method) AND TITLE-ABS-KEY (“anti-tubercul*”) OR TITLE-ABS-KEY (antitubercul*) OR TITLE-ABS-KEY (“TB drug*”) OR TITLE-ABS-KEY (“TB regimen”) AND TITLE-ABS-KEY (Urin*)

Search strategy for Web of Science:

(((((((KP=(HPLC))ORKP=(“highperformanceliquidchromatography”)) OR KP=(“analytical method*”)) AND KP=(“anti-tubercul*”)) OR KP=(“antitubercul”)) OR KP=(“TB drug*”)) OR KP=(“TB regimen”)) AND KP=(urine)) AND KP=(“mycobacterium tuberculosis”)

RESULTS

The authors identified 296 potential articles from different electronic databases (PubMed=248, Scopus=44, and Web of Science=4) and four articles from other sources such as Google Scholar and ResearchGate. After removing duplicates, 274 articles were examined and, after initial screening based on title and abstract, 242 articles were removed. The authors then independently assessed the full texts of 32 potentially relevant articles and excluded 19 articles, of which 15 were published before 1998, the full text was not available for two articles, and the full text of the remaining two articles was in Korean language. The authors identified 13 articles for potential inclusion [6,10-21]. The authors have presented a flow diagram detailing the selection of articles in [Table/Fig-1].



[Table/Fig-1]: PRISMA flow diagram.

Of the included 13 articles, two articles were clinical trials performed in Thailand [10] and Japan [11], and the others [6,12-19] were original research articles. Five articles [6,15,16,18,19] were from India, and the others were from China [20], Finland [21], Hong Kong [12], Indonesia [17], Japan [11], Russia [14], Spain [13], and Thailand [10]. Among the 13 articles, 11 articles [6,11-18,20,21] utilise urine samples from active TB patients who were on the anti-TB regimen, and two articles [19,21] include urine samples from a healthy volunteer and then spiked the urine with anti-TB drugs. Among the included articles, one article [10] included the urine sample from children, whereas the other articles did not specify the age group. Methods developed by Hemanth A et al., in 2004 [15] and in 2014 [16] for rifampicin and isoniazid, respectively, and by Mishra et al., in 2019 [6] for rifampicin and in 2018 [18] for isoniazid had very simple mobile phase compositions and easy sample extraction procedures. All the included articles [6,10-21] estimated the levels of anti-TB drugs in urine samples using HPLC-based methods. The participants included in this review were on an anti-TB regimen. In this review, we summarised articles published between 1998 and 2023 and extracted data on mobile phase composition, chromatographic parameters, and sample preparation in one table. The authors summarised 13 included articles in [Table/Fig-2] [6,10-21].

DISCUSSION

One of the pivotal health targets outlined in the United Nations Sustainable Development Goals (SDGs) is to end the TB epidemic by the year 2030 [2]. However, this ambitious goal faces mounting challenges from the emergence of MDR-TB and XDR-TB, which pose significant threats to the effectiveness of anti-TB programs. Although TB is curable through timely and comprehensive treatment lasting 6 to 9 months, achieving successful outcomes is hindered by several factors. High treatment default rates, premature discontinuation of therapy, non adherence to prescribed medications, and inadequate awareness about the disease collectively contribute to suboptimal results in TB treatment [8]. This results in drug-resistant (MDR, XDR) TB. The insights of Sir John Crofton, a pioneering figure in TB treatment, resonate profoundly with the current challenges. According to Sir John Crofton, “The greatest disaster that can happen to a patient with TB is that these organisms become resistant to two or more standard drugs. The development of drug

S. No.	Author and year of study	Study design and settings	Population	Mobile phase used	Chromatographic parameters	Sample treatment	Comments
1.	Phaisal W et al., 2022 [10]	Clinical pharmacokinetic study (TCTR20190423002) Thailand	Young children with LTBI n=12	For INH: acetonitrile and 0.2% TEA in 20 mM of NaH_2PO_4 at pH 7 (5:95) For RPN: ACN and 0.2% TEA in 20 mM of NaH_2PO_4 at pH 4 (50:50)	1. Chromatographic column: C18 2. Flow rate: NR 3. Detection wavelength: 267 (INH) and 238 (RPN) nm 4. Injected volume: NR 5. Run Time: 15 min	Sample protein precipitation: For INH: 0.1 M sodium acetate and MeOH (1:1) (Refer to Oliveira MAL et al., [22]). For RPN: MeOH protein precipitation. (Refer to Hemanth Kumar AK et al., [15]).	Further study in a larger cohort is required
2.	Mishra P et al., 2019 [6]	Original Research India	TB patients n=NR	0.15 M SDS, 6% 1-pentanol, and 0.01 M phosphate buffer at pH 7	1. Chromatographic column: C18 2. Flow rate: 1 mL/min 3. Detection wavelength: 337 nm 4. Injected volume: 20 µL 5. Run time: 15 min	1:5 (v/v) diluted in MP, vigorously shaken, filtered and directly injected in column.	Inexpensive, easy-to-handle, one-step, rapid and reproducible, without tedious extractions
3.	Mishra P et al., 2018 [18]	Original Research India	TB patients and healthy volunteers TB=NR Healthy V.=6	0.15 M SDS-12.5% 1 propanol, 0.01 M Na2HPO4 at pH 7	1. Chromatographic column: C18 2. Flow rate: 1 mL/min 3. Detection wavelength: 265 nm 4. Injected volume: 20 µL 5. Run time: 5 min	1:5 (v/v) diluted in MP, vigorously shaken, filtered and directly injected in column.	A simple, reliable, cost-effective, and rapid method
4.	Lily et al., 2018 [17]	Original Research Indonesia	TB patients n=NR	65:35 v/v MeOH: 0.01 M sodium phosphate buffer pH 5.2	1. Chromatographic column: C18 2. Flow rate: 0.8 mL/min 3. Detection wavelength: 254 nm 4. Injected volume: 5 µL 5. Run time: 3.01 min	Method modified from the Panchagnula R et al., [19] 1999. Samples were vortexed for 15s. Cfgat 10.000 rpm for 15 min. Filter the solution directly into the HPLC vial. Sonicated for 15 min, then injected into HPLC.	Short sample processing and requires less urine volume (500 µL)
5.	Pynnönen ST and Tuhkanen TA 2014 [21]	Research Article Finland	Healthy volunteer's urine was spiked with antiviral and antibiotics. n=NR	MP consisted of two parts: Eluent A: Acetonitrile Eluent B: 10 mM KH2PO4 (pH 2.5) Gradient elution	1. Chromatographic column: Kinetex XB-C18 2. Flow rate: 2.5 mL/min 3. Detection wavelength: 210 nm to 264 nm 4. Injected volume: 5 µL 5. Run time: 6 min	SPE: 1 mL MeOH+1 mL miliQ water+1 mL urine sample. Elution 1: Dried the solvent for 1 min, eluted with 1 mL 2% NH_4OH to collect 3TC, and then vacuum dried for 1 min. Elution 2: 1 mL of ACN/MeOH/acetic acid (50:50:2 v/v/v) to collect four antibiotics. 1 mL/min flow rate and collect in amber-coloured vials.	New core-shell column technology to screen antivirals and antibiotics simultaneously
6.	Hemanth Kumar AK et al., 2014 [16]	Research Article India	TB patients n=NR	Water and MeOH mixed in a ratio of 80:20 v/v	1. Chromatographic column: C8 2. Flow rate: 1.2 mL/min 3. Detection wavelength: 274 nm 4. Injected volume: 100 µL 5. Run time: 6 min	Filtered samples were diluted 1:25 in Milli Q water and directly injected into the column.	The simple MP, a rapid, accurate, and reproducible method with no sample pretreatment
7.	Ming ZZ et al., 2009 [20]	Research Article China	TB patients n=NR	For INH: ACN: water (5:95, v/v) For INZ: ACN: water (50:50, v/v)	1. Chromatographic column: LiChrospher C18 2. Flow rate: 0.8 mL/min (INH) and 1 mL/min (IHZ) 3. Detection wavelength: 264 nm (INH) and 365 nm (IHZ) 4. Injected volume: 20 µL 5. Retention Time: 2.1 min (INH) and 3.7 min (IHZ)	For INH: 5 mL of urine samples were diluted to 50 mL with distilled water filtered through 0.45 µm membrane and then treated according to the CPE.	The CPE technique is simple and more environmentally friendly since it does not use hazardous organic solvents
8.	Hemanth Kumar AK et al., 2004 [15]	Research Article India	TB patients n=NR	0.05 M phosphate buffer (pH 2.6): ACN (55:45 v/v) at 254 nm	1. Chromatographic column: C18 2. Flow rate: 1.2 mL/min 3. Detection wavelength: 254 nm 4. Injected volume: 20 µL 5. Run Time: 12 min	Mix the sample with 1.0 mL of citrate-phosphate buffer (1.5 M, pH 7.0) and extract with 2.0 mL chloroform by vortexing for 1 min, cfg for 10 min at 2500 rpm. Discard the aqueous layer and evaporate the chloroform layer at 600 C. Reconstitute the residue in 500 µL MP.	Linear, sensitive and selective method for the broad range of concentrations for RMP and DRMP

9.	Espinosa-Mansila A 2002 [13]	Research Article Spain	TB patients n=NR	0.02 M potassium dihydrogen phosphate pH 7.0 buffer, a 5% (v/v) content of MeOH for 1 min, an 8% (v/v) content of MeOH for 3.4 min, and a 75% (v/v) content of MeOH for 4 min	1. Chromatographic column: C18 2. Flow rate: 1 mL/min 3. Detection wavelength: 254-475 nm 4. Injected volume: 20 µL 5. Run time: 8 min	4 mL urine diluted to 100 mL (dilution 1:25) with a 0.02 M potassium dihydrogen phosphate buffer of pH 7.0.	Simple, and a short time, less than 8 min. No sample pretreatment is required
10.	Hashiguchi M et al., 2002 [11]	Clinical Trial Japan	TB patients and Healthy volunteers TB: 278 Healthy: 4	60:40 (v/v) phosphate buffer (0.01 M, pH 2.5) MeOH containing 0.01 M SDS	1. Chromatographic column: C18 (LC column ODS) 2. Flow rate: 1 mL/min 3. Detection wavelength: 267 nm 4. Injected volume: 25 µL 5. Run time: NR	Dilute urine samples 1:10 in distilled H_2O. Add 50 µL of the iproniazid phosphate solution (20 µg/mL) as an internal standard, + 500 µL of phosphate buffer (0.5 M, pH 7.4)+0.5 g NaCl to 200 µL of the diluted urine. As the extracting solvent, shake for 10 min with 1 mL of ethyl acetate/1-butanol (7:3, v/v) solution. After the mixture was cfg for 3 min at 15,000×g, 800 µL of the organic layer was transferred into a new microtube. Add 800 µL of the extracting solvent to the remaining aqueous layer. After mixing for 10 min, cfg at 15,000×g for 3 min. Combine the second 800 µL of the organic layer with the first organic layer, and then add 300 µL of phosphate buffer. Shake for 10 min and cfg for 2 minutes at 15,000×g. Take 200 µL of the aqueous layer and inject 25 µL into the HPLC system.	Not reported
11.	Panchagnula R et al., 1999 [19]	Research Article India	Healthy volunteers' urine samples spiked with anti-TB drugs n=NR	MeOH–sodium phosphate buffer (pH 5.2; 0.01 M) (65:35, v/v)	1. Chromatographic column: RP Nova-Pak C18 2. Flow rate: 1 mL/min 3. Detection wavelength: 254 4. Injected volume: 50 µL 5. Run time: 17 min	Dissolve vacuum-dried anti-TB drugs in 5 µL MeOH+95 µL urine and vortex 1 min. Add 500 µL of MeOH and vortex for 3 min, followed by cfg at 10,000 rpm for 15 min. Vacuum-dried 300 µL of supernatant dissolved in 500 µL MP and 50 µL injected in the column.	Low volumes of urine samples, simple and fast extraction procedure. Simple MP and single detection wavelength (254 nm).
12.	Chan CY et al., 1998 [12]	Research Article Hong Kong	TB patients n=193	MeOH: ACN:0.4 M citric acid (3:1:10 v/v)	1. Chromatographic column: C18 2. Flow rate: 1 mL/min 3. Detection wavelength: 208 to 340 nm 4. Injected volume: 10-50 µL minimum volume 5. Run time: 5-15 min depending on the quinolone being assayed	Extraction procedure was taken from author's previous publication [23]. 0.1 mL samples were deproteinated by adding an equal volume of 10 M perchloric acid. Shaken and incubate the mixture at 55°C for 15 min and then cfg at 10,000 g for 5 min. 10-50 µL clear supernatant was injected into the HPLC system.	Simple reagents with a basic HPLC system 20 min sample preparation and run time between 5 to 15 min
13.	Evgen'ev MI, 1998 [14]	Research Article Russia	TB patients n=26	For INH: ACN: 3 mM acetate buffer (pH 5.5) (25:75, v/v) For DPPAH: ACN:5 mM acetate buffer (pH 5.5):MeOH (28:67:5, v/v)	1. Chromatographic column: Silasorb C-18 (10 mm) and Silasorb 600 (10 mm) 2. Flow rate: 0.1 mL/min 3. Detection wavelength: 510 nm (INH) to 495 nm (DPPAH) 4. Injected volume: 4 µL volume 5. Run time: Not mentioned	2 mL urine is diluted to 50 mL with distilled H2O. 1 mL of 0.01 M DNBF solution in ACN and 2 mL of 0.05 M acetate buffer solution (pH 5.5) areadded to an aliquot (8 mL) of the resulting solution mentioned above. After stabilisation for 5 min, an aliquot of 4 µL was injected into the LC system.	This method allows authors to diagnose the biotransformation phenotype of xenobiotics by acetylation rate

[Table/Fig-2]: Characteristics of included articles [6,10-21].

LTBI: Latent tuberculosis infection; INH: Isoniazid; RPN: Rifapentine; MeOH: Methanol; TEA: Triethylamine; MP: Mobile phase; TB: Tuberculosis; SPE: Solid phase extraction; ACN: Acetonitrile; INZ: Isonicotinylhydrazone; CPE: Cloud point extraction; RMP: Rifampicin; DRMP: Desacetyl rifampicin; SDS: Sodium dodecyl sulfate; RP: Reversed phase; cfg: Centrifuge; DPPAH: Diphenylphosphinylacetic hydrazide; DNBF: 5,7-dinitrobenzofurazan

resistance may be a tragedy for the patient and others, as he can infect other people with his drug-resistant organisms [24]."

According to the existing literature, different approaches such as liquid chromatography-based methods, colorimetry, spectrophotometry, and others are available for assessing treatment adherence and therapeutic drug monitoring in patients' biological fluids, such as blood and urine [9]. However, for the given purpose, HPLC-based methods are considered the gold standard [9]. Several articles have reported that HPLC is frequently used to determine the presence of anti-tubercular drugs in various biological fluids and formulations or matrices for quality control [6]. This review included HPLC-based articles with simple mobile phases and easy extraction procedures.

The authors also encountered some old HPLC-based methods [25-38] that were published before 1998. However, we did not include those articles due to limited access to full texts, complicated mobile phase compositions, and extensive sample preparation methods. TB treatment is long and consists of the initial and continuation phases. The initial or intensive phase involves administering four drugs (isoniazid, rifampicin, ethambutol, and pyrazinamide) for two months. This phase is followed by a continuation phase of either four months with two drugs (isoniazid and rifampicin) or six months with two drugs (isoniazid and ethambutol) when ensuring adherence to rifampicin treatment is not possible. Since the treatment duration is extended, regular and uninterrupted intake of drugs is of utmost importance to prevent the development of drug resistance in TB [4]. To overcome this challenge, routine drug monitoring and checking treatment adherence are necessary. Therefore, the development and adoption of HPLC-based methods with simple mobile phase and easy sample extraction are required. These methods can be quickly implemented in clinical laboratories as standard analytical tools for monitoring treatment adherence in patients undergoing DOTS therapy and for adjusting their future therapeutic doses [6].

Strengths and Limitation(s)

This review focused on drug estimation in urine samples because collecting urine samples offers several advantages over blood or other biological fluids. For example, urine collection provides a low-cost point-of-care testing alternative with minimal processing to quantify drug excretion [39]. Urine collections are non-invasive and particularly suitable for all groups, especially paediatric patients [16]. Urine is a chemically complex and readily accessible biological fluid, and urinary biomarkers have recently been used as diagnostically relevant markers of infectious diseases and prognostic markers of treatment efficacy [40]. Additionally, collecting plasma samples from paediatric patients is not recommended when a substantial volume and multiple samples at different time intervals are necessary.

The authors found very few HPLC-based methods for urine samples compared to methods developed for plasma. The limitation of this review is that urine samples are prone to contamination. Proper collection and storage practices are recommended when handling urine samples. The use of boric acid-coated leakproof vials is advised for prolonged storage of urine samples to prevent contamination. Additionally, after collection, the urine sample should be stored at 4°C until use.

CONCLUSION(S)

In conclusion, this review focused on HPLC-based methods for determining anti-tubercular drugs in urine samples to assess treatment adherence. HPLC-based methods are considered the gold standard and are known for their simplicity and sensitivity in drug analysis. While there are other MS/MS-based methods available, they tend to be expensive and require laborious extraction procedures involving large volumes of hazardous volatile organic solvents. The authors specifically focused on urine samples because they are easily obtainable from individuals of all ages and require low-cost extraction, primarily through dilution. The HPLC-based methods from the past 25 years were included to provide a comprehensive overview of different mobile phases and extraction procedures in one framework.

Two articles by Hemanth Kumar AK in 2004 and 2014 reported simple mobile phases and sample preparation methods for estimating rifampicin and isoniazid [15,16]. Additionally, articles published in the last five years have demonstrated the use of simple mobile phases with minimal or no extraction procedures, making them easily implementable in resource-poor settings. This review highlights the need for more simple and rapid HPLC-based methods to assess treatment adherence in TB patients in resource-poor settings.

Author contributions: SU proposed the concept of the review and developed and executed the search strategies. SU and AD screened the titles, abstracts, and full-text articles, with SZQ resolving any discrepancies through discussion. SU drafted the manuscript, and all authors contributed significantly to this review through reading, writing, and revision.

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PARTICULARS OF CONTRIBUTORS:
1. Research Associate, Department of Research and Development, Datta Meghe Institute of Higher Education and Research, Wardha, Maharashtra, India.
2. Professor and Head, Department of Biochemistry, Datta Meghe Institute of Higher Education and Research, Wardha, Maharashtra, India.
3. Director, Department of Research and Development, Datta Meghe Institute of Higher Education and Research, Wardha, Maharashtra, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Shilpa Manish Upadhyay,
Research Associate, Department of Research and Development, Datta Meghe Institute of Higher Education and Research, Wardha-442004, Maharashtra, India.
E-mail: shilpstrivedi@gmail.com

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