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Liquid Chromatographic Methods for Anti-tubercular Agents: An Overview

Deepika Bairagee[1]

[1]Oriental College of Pharmacy and Research, Oriental University, Indore, Madhya Pradesh, India

Abstract : The aim of this review is to summarise the different analytical and bioanalytical methods used to determine the concentration of anti-tubercular agents from last few years. As we know that tuberculosis is a life threatening disease and second to HIV in terms of deaths due to infectious diseases. Drug resistance development of the first-line drugs is a most important concern in the cure of this disease. There is no comprehensive and critical review in the literature for the analytical and bioanalytical methods for the determination of ant-tubercular agents from last few years. So this work provides the detailed account on the chromatographic methods reported in the literature for the estimation of various ant-tubercular drugs.
Keywords : Anti-tubercular drugs; Chromatographic methods; Bioanalytical methods; Estimation; Concentration.

Introduction:

Tuberculosis is one of the oldest main killers between infectious diseases since ancient times. There have been several reports on TB from different parts of the world among various civilizations. *Mycobacterium tuberculosis*, is the caustive agent behind TB[1,2]. Two major forms of TB have been known on the basis of site of infection, called as Pulmonary Tuberculosis (PTB) and Extra Pulmonary Tuberculosis (EPTB). Pulmonary TB is the most common form of the disease and constitutes about 85% of all TB cases[3,4].

Mycobacterium tuberculosis is carried in airborne particles, called droplet nuclei, of 1-5 microns in diameter and is transmitted when a perosn with active TB disease of the lungs or throat cough/sneezes[5]. People nearby may breathe-in these bacteria and become infected. Human beings are infected through the respiratory tract and the tubercle bacilli spreads by lymphatic system and blood stream to different organs. the course of tubercular disease is divided into primaty pulmoanry TB, latent TB infection and acitve TB disease, including tuberculo-meningitis, skin TB, scrofulous TB, pleurisy TB, cordis TB, urinary systemic TB, digestive systemic TB, skeletal TB, etc[6-9]. People with active TB disease can e treated and cured if they seek medical help. Even better, people with latent TB infection can take medicine so that they will not develop active TB disease[10]. Various targets were explored for antitubercular drug discoveries, recently explored DprE1 is one of them[11-14]. Few analogues of DprE1 inhibitors are inder pre clinical trials[15].

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People having active tuberculosis disease develop symtoms, like cough, which propels the bacteria into air, where these bacilli can be inhaled by others. Therfore, TB must be diagnosed and treated as soon as possible to render the person non-infectious and prevent the spread of the disease. The most common methods to diagonise tuberclosis is a simple skin test, though blood tests are becoming more commonplace. A small amount of a substance called PPD tuberculin is injected just below the skin of your inside forearm. You should feel only a slight niddle prick. Within 48-72 hours, a health care professional will check your arm for swelling at the injectin site. A hard, raised red bump means, you are likly to have TB infectio. The size of the bump determines whether the test results are significant[16].

The first line drugs are used in the treatmet of new TB cases in which the risk of resistance is low or negligible and usually are given orally, while the second line drugs are used to treat cases, whihc are resistnat to first line drugs. The second line drugs can be further categorized into 4 subgroups, viz, injectable second lne drugs, fluoroquinolones, oral bacteriostatic anti-TB agents and anti-tuberculosis agents. The main first line drugs used are Isoniazid, Rifampicin, Ethambutal and Streptomycin[17-19].

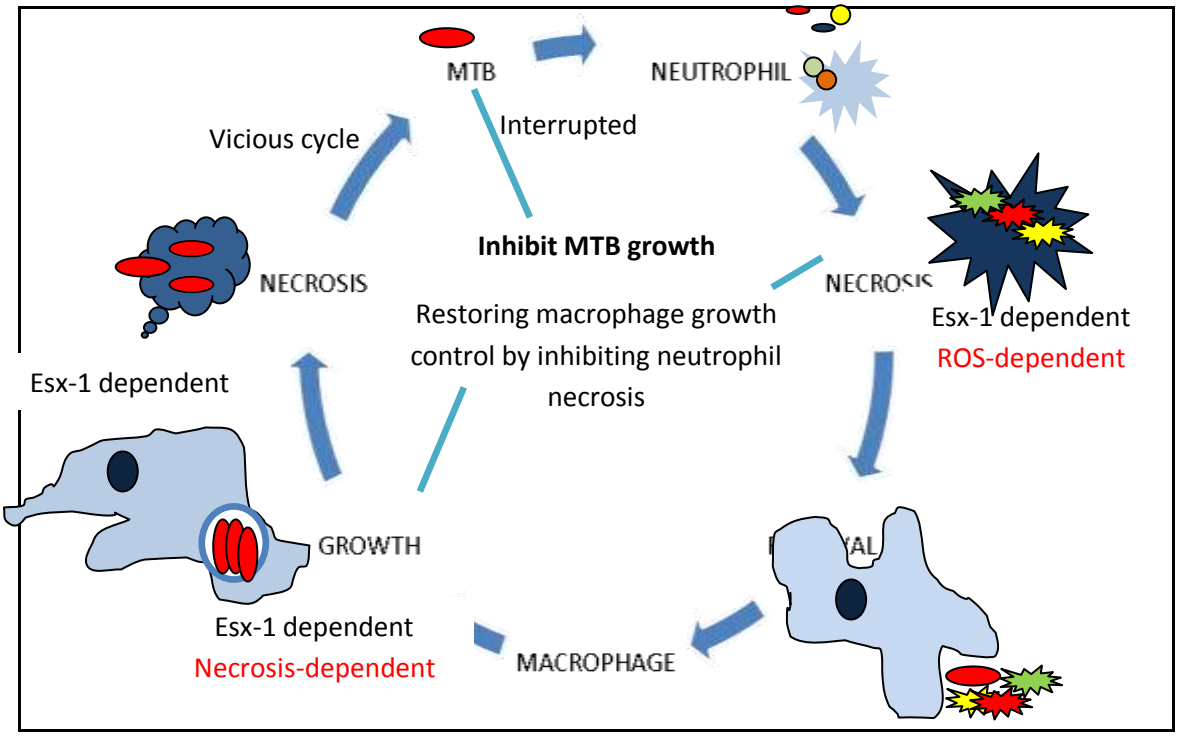

Fig. 1. The vicious circle of host cell necrosis during MTB

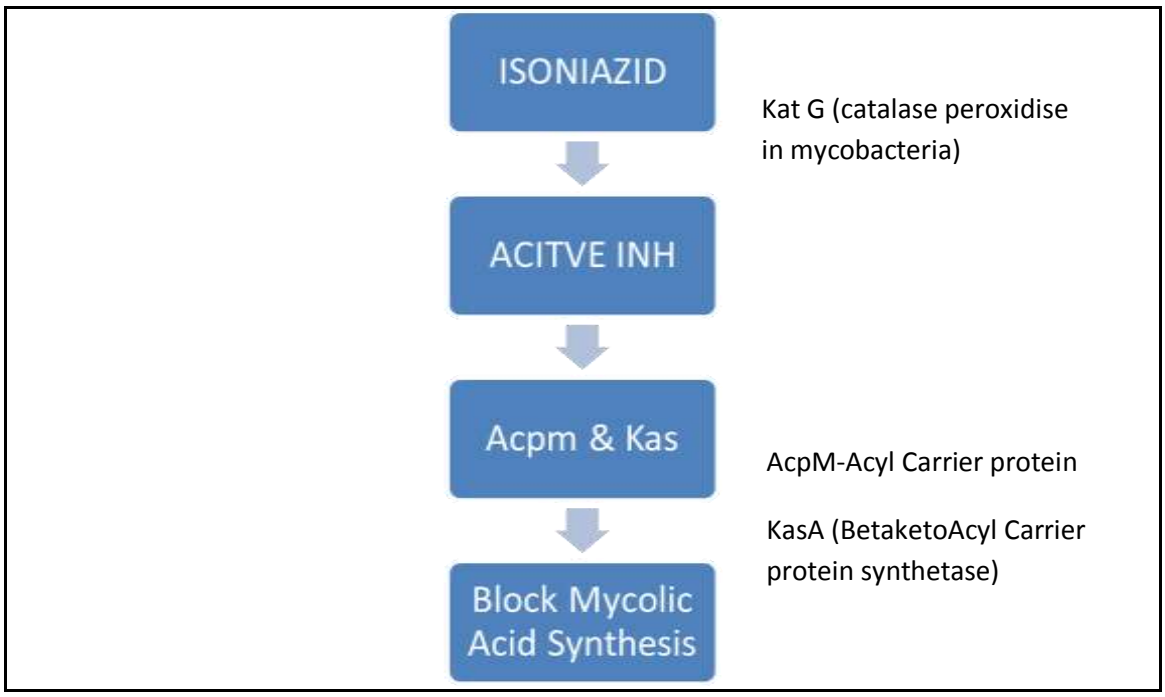

Fig.2. Mechanism of action of anti- tuberculin drug

As we know that anti-tubercular drugs plays a very important role in today's scenario. So it's necessary to perform the various analytical methods and bioanalytical methods on them. According to literature review of last few years there are so many precise and practical analytical methods are used for the fast qualitative analysis of pharmaceutical active substances.

In 1992, *Shah Y et. al.* a liquid chromatographic procedure for the analysis of Rifampicin and Isoniazid in pharmaceutical dosage forms utilizing reverse phase chromatography was developed. Isolation of analytes was carried out under isocratic conditions with an octadecylsilane column and an aqueous mobile phase containing methanol (75%) and 0.02M Disodium Hydrogen Orthophosphate (25%) with pH 4.5 adjusted with orthophosphoric acid. The detection was done at 254 nm[20].

In 2002, *Espinosa-Mansilla A et. al.*, the simultaneous determination of the antitubercular drugs rifampicin, pyrazinamide, isoniazid and the acetylisoniazid metabolite has been accomplished by LC, using a C-18 analytical column. The chromatographic method uses a gradient flow in three steps, in conjunction with a programmed diode array photometric detection. In a 0.02M potassium dihydrogen phosphate pH 7.0 buffer, a 5% (v/v) content of methanol for 3.4 min, and a 75% (v/v) content of methanol for 4 min were used. At 4.5 min, the wavelength value of detection was changed from 254 to 475nm. Creatinine, acetylisoniazid, isoniazid and pyrazinamide were eluted in the first 4.5 min and rifampicin before 8 min. The method has been satisfactorily applied to the determination of the drugs in urine samples and in pharmaceuticals. The proposed LC method is simple, and a short time, less than 8 min is necessary for compounds elution[21].

In 2002, *Naidong W et. al.* demonstrated a novel approach of eliminating these two steps in 96-well SPE by using normal-phase LC/MS/MS methods with low aqueous/high organic mobile phases, which consisted of 70-95% organic solvent, 5-30% water, and small amount of volatile acid or buffer. While the commonly used SPE elution solvents i.e. acetonitrile and methanol have stronger elution strength than a mobile phase on reversed-phase chromatography, they are weaker elution solvents than a mobile phase for normal-phase LC/MS/MS and therefore can be injected directly. Analytical methods for a range of polar pharmaceutical compounds, namely, omeprazole, metoprolol, fexofenadine, pseudoephedrine as well as rifampin and its metabolite 25-desacetyl-rifampin, in biological fluids, were developed and optimized based on the foregoing principles[22].

In 2003, *Mohan B et. al*, observed that the requirements of theoretical plates listed in the given method were met for rifampcin, but not for isoniazid and pyrazinamide, even on columns of different makes. The resolving power of the method was also dependent upon makes of the column. On two of the three columns of

the three tested, it was able to resolve most degradation products, except rifampicin N-oxide and 25-desacetylrifampicin, which were overlapping. The method was modified and an overall satisfactory resolution for all components was obtained by changing the buffer: organic modifier ratio of solution B in the gradient from 4:55 to 55:45 and decreasing the flow rate from 1.5 to 1.0 ml/min, keeping all other conditions constant[23].

In 2004, *Hemanth Kumar AK et. al.*, developed a HPLC method for the determination of RMP and DRMP in plasma and urine. Separation in both was achieved by reverse phase chromatography on a C18 column with a mobile phase composition of 0.05 M phosphate buffer: acetonitrile (55:45 v/v) at 254 nm. The retention times of DRMP, RMP and RPN, the internal standard was 2.9, 4.8 and 10.5 min respectively. The assay was linear from 0.25 to 15.0 µg ml^{-1} for urine. Both intra-day and inter-day accuracy and precision data showed good reproducibility[24].

In 2007, *Song SH et. al.* described a method that can rapidly and simultaneously measure the blood concentration of four anti-tuberculosis drugs i.e. isonizid, rifampicin, pyrazinamide and ethambutol and two major metabolic rations like acetylisoniazid/isoniazid and 25-desacetylrifampicin/rifampicin using high-performance liquid chromatography/tandem mass spectrometry (HPLC/MS/MS). A C18 reversed-phase column and gradients of methanol in 0.3% formic acid and water were used for HPLC separation. The drug concentrations were determined by multiple reactions monitoring in positive ion mode and the assay performance was evaluated. The preparation of 20 samples including two steps of deproteinization with 50% and 100% methanol was performed within 20 min and chromatographic separation was achieved within 4 min/sample. Interassay calibration variability data obtained over concentrations of 0-8 microg/mL for isoniazid and ethambutol and 0-80 microg/mL for rifampicin and pyrazinamide showed a linear and reproducible curve. Within-run and between-run imprecision were 1.9-5.5% and 3.5-10.5% and the lower limits of detection and quantification were 0.01-0.5 microg/mL and 0.05-1.0 microg.mL, respectively[25].

In 2007, *Patel PM et. al*. developed a method for determination of Rabeprazole in pharmaceutical bulk dosage form. The method was based on the formation of ion-pair complexes of the drug with four dyes, viz. bromo thymol blue, bromocresol green, bromophenol blue and bromocresol purple in acidic buffer solutions followed by their extraction in chloroform. The absorbance of the organic layer was measured at its respective wavelength of maximum absorbance against the corresponding reagent blank. The method has been statistically evaluated and was found to be precise and accurate. Phosphate buffer of pH 2 and bromocresol green dye gave maximum absorbance of Rabeprazole at 454 nm[26].

In 2009, *Dhal SK et. al.* developed a method for simultaneous determination of pyridoxine hydrochloride, isoniazid, pyrazinamide and rifampicin in a tablet dosage form. Chromatographic analysis was performed on a 250 X 4.6 mm I.D. C18 column packed with 5 mm in size particles applying gradient elution with a mobile phase composed of acetonitrile and 15 mmol/L potassium dihydrogen phosphate buffer of pH adjusted to 4.0±0.1 with 0-phosphoric acid. The ratio was 11:89v/v for the initial 4.5 min, and then it was maintained at 50:50 v/v; the flow rate was 1mL/min. UV detection was performed at 235 nm. The total run time was 20 min. Retention times for pyridoxine hydrochloride, isoniazid, pyrazinamide and rifampicin were 3.687, 4.113, 5.041 and 12.829 min, respectively. Limits of detection were 0.043, 0.063, 0.036 and 0.059 µg/mL and limits of quantification were 0.13, 0.19, 0.11 and 0.18 µg/mL for pyridoxine hydrochloride, isoniazid, pyrazinamide and rifampicin respectively[27].

In 2010, *Mulabagal V, et. al.*, developed an ultrafiltration-liquid chromatography/mass spectrometry (UF-LC/MS) ligand based binding assay and an LC/MS based functional assay for Mycobacterium tuberculosis shikimate kinase (MtSK) were developed. Compounds 1,2,3 and 4 were tested for MtSK (1µM) at a concentration of 1µM. In order to evalutate the MtSk inhibitory activity, compounds 1-4 were tested at concentrations ranging from 0.05 to 1µM and the enzymatic activity was assessed by quantifying shikimate-3-phosphate by LC/MS after 60min incubation with 2mM shikimic acid as a substrate. The EC_{50} values of compounds 1,2,3 and 4 were 0.30, 0.24, 0.07 and 0.18 µM, respectively. The ligands and the S3P were analyzed using positive and negative electrospray LC/MS, respectively. The calibration curve for S3P was prepared with concentrations ranging from 4 to 125µg/mL, and the lower detection limit (LOD) of S3P was identified as 1.95µg/mL[28].

In 2010, *Zhou Z et. al.*, developed a method for the simultaneous determination of pyrazinamide (PZA), isoniazid (INH), rifampicin (RFP) and acetylisoniazid (AcINH) in human plasma. Separation was performed on a Max-RP C_{12} column using gradient elution and a flow-rate program. The mobile phase was methanol-

acetonitrile-buffer (20 mM of heptanesulfonic acid sodium, pH 2.5) with a ratio of 10:8:82 (v/v/v) at the initial phase. All calibration curves had good linearity (r^2 >0.99) between the test ranges. The intra-and inter-day precision was less than 8.8% in good accuracy (<15%). The limit of detection with a signal-to-noise (S/N) of 3 was 0.014, 0.009, 0.023 and 0.054 µg/mL for PZA, AcINH, INH and RFP, respectively[29].

In 2012, *Bhandari R. et.al*, developed a reverse phase high performance liquid chromatography method for quantitative determination of isoniazid (INH) in plasma, brain, liver and kidney samples and in solid lipid nanoparticles (SLNs). Isoniazid was analyzed by using a reverse phase column (Waters, Symmetry shield RP-18, 4.6mm X 150 cm, 5 microns), with mobile phase consisting of 0.1 M phosphate buffer, pH 5 (pH adjusted with ortho phosphoric acid) and methanol and the detection was made at 254 nm using Photo Diode Array detector at a temperature of 30˚C (sample 4˚C). The retention time for INH was around 3.5 minutes. The calibration curves were linear (r^2= 0.9998) over a concentration range from 250ng to 25,000 ng/mL. Limit of detection was 150ng/mL and the limit of quantitation was 200ng/mL for plasma and tissue homogenates. Intra and inter-day variability's (RSD) for extraction of INH from plasma and other tissue homogenates were less than 5% and accuracy was ± 5%. The results established selectivity and suitability of the method for pharmacokinetic studies of INH for INH SLNs[30].

In 2012, *Siddhartha TS et. al.*, developed a HPLC method for the determination of rifampicin (RIF) by UV detection in human plasma. Rifampicin was extracted from plasma utilizing liquid-liquid extraction process using a 70:30% v/v mixture of t-butyl methyl and dichloromethane. Hydrochlorothiazide (HCTZ) was used as internal standard. Both RIF and HCTZ were eluted under isocratic mode using a 150 X 4. 6mm i.d., 5µm Phenomenex ODS 2 C18 coloumn. A mixture of 40:60% v/v acetonitrile and 10mM potassium dihydrogen phosphate was used as mobile phase at a flow rate of 1.0ml/min. The retention times for RIF and HCTZ were 6.80 and 2.56 minutes, respectively. The method showed good linearity in the range of 0.31 to 25.48 µg/mL. The recovery of rifampicin was 90.07% with a CV of 3.26% and recovery of internal standard was 91.24% with a CV of 2.31%. A rapid, sensitive, simple and cost effective method for the estimation of rifampicin in human plasma was developed[31].

In 2012, *Shewiyo DH et. al.*, developed a new RP-HPTLC method for the separation of pyrazinamide, isoniazid, rifampicin and ethambutol in a four fixed-dose combination tablet formulation. It is a single method with two steps in which after plate development pyrazinamide, isoniazid and rifampicin are detected at an UV wavelength of 280nm. Then ethambutol is derivatized and detected at a VIS wavelength of 450 nm. Methanol, ethanol and propan-1-ol were evaluated modifiers to form alcohol-water mobile phases. Systematic optimization of the composition of each alcohol in the mobile phase was carried out using the window diagramming concept to obtain the best separation. Examination of the Rf distribution of the separated compounds showed that separation of the compounds with the mobile phase containing ethanol at the optimal fraction was almost situated within the optimal Rf-values region of 0.20-0.80. Therefore, ethanol was selected as organic modifier and the optimal mobile phase composition was found to be ethanol, water, glacial acetic acid (>99% acetic acid) and 37% ammonium solution (70/30/5/1, v/v/v/v). The method is new, quick and cheap compared to the actual method in the International Pharmacopoeia for the assay of the 4FDC tablets, which involves the use of two separate HPLC methods[32].

In 2013, *Siddhartha TS et.al.*, developed a extraction process which involved a liquid-liquid extraction using 70:30 % v/v mixture of t-butyl methyl ether and dichloromethane. Both pyrazinamide and the internal standard were eluted under isocratic mode using a 150X4.6 mm i.d., 5µm Phenomenex ODS 2 C_{18} column. The mobile phase was composed of a mixture of 15:85% v/v methanol and O mM potassium dihydrogen phosphate at a flow rate of 1.0 ml/min. The wavelength of detection was 268 nm. The injection volume was 20µL. The runtime of the method was 8min. The method showed good linearity in the range of 1.02-50.23 µg/mL. The overall recovery of pyrazinamide was 27.21% with a CV of 2.71% and recovery of internal standard was 83.34% with a CV of 4.38%[33].

In 2014, *Shah UM, et. al.*, used methanol and distilled water as diluent. The wavelength selected for the analysis was 262nm, 338nm and 477nm for INH, PIPE and RIFA respectively. The second RP-HPLC method has been developed using Acetonitrile as diluent. Sucessful separation of drugs was achieved on LC18 100A˚ column (250 X 4.6 mm, 5µ) using 0.01M Sodium Dihydrogen Orthophosphate, pH 6.5 and acetonitrile (40:60, % v/v) as mobile phase with flow rate of 0.9mL/min. The wavelength of detection was 282 nm. Validated of developed methods was done according to ICH Q2 (R1) guidelines. Calibration curve was linear over the concentration range of 12-34.5 µg/mL (INH), 8-23 µg/mL (RIFA) and 0.4-1.15 µg/mL (PIPE) respectively or

absorption correction method and 30-330µg/mL (INH), 20-220 µg/mL (RIFA) and 1-11µg/mL (PIPE) for RP-HPLC method with r^2 value greater than 0.995. Accuracy of methods was determined by recovery studies and it was found to be 98 to 102%. The % RSD values for all the validation parameters were less than 2.0% for both the methods[34].

In 2015, *Bajetto L et.al.,* extracted Ethambutol, isoniazid, pyrazinamide and rifampicin from plasma and PBMCs using two separate and optimized procedures; analysis was performed using UPLC coupled with a mass-mass detector system (UPLC-MS-MS). Antitubercular levels in patients were assayed at the end of the dosing interval and 2 h post-dose. The method was accurate and precise. Recovery and the matrix effect were reproducible. While rifampicin intracellular concentrations were similar to plasma values isoniazid and pyrazinamide intracellular concentrations were lower than plasma values and ethambutol intracellular concentration were significantly higher than plasma values[35].

In 2015, *Sturekenboom MG, et. al.,* developed a LC-MS/MS method for the quantification of isoniazid, pyrazinamide and ethambutol. Stable isotope-labelled isoniazid-D4 and ethambutol was investigated and proved low. Therefore, sample preparation using ultrafiltration could be applied, resulting in linear calibration curves in the range of 0.2-8 mg/L for isoniazid and ethambutol and 2-80mg/L for pyrazinamide. The method was validated according to the guidelines of the FDA. A fast, simple and reliable LC-MS/MS method has been developed for the simultaneous determination of isoniazid, pyrazinamide and ethambutol in human serum for therapeutic drug monitoring and pharmacokinetic studies[36].

In 2015, *Chellini PR, et. al.,* developed an HPLC-diode array detector method for the determination of RIF, INH, PYZ, and EMB in fixed dose combination tablets. Chromatographic experiments were performed on an Agilent 1200 HPLC system, and the separation was carried out on Purospher STAR RP 18e (250X4.6 mm id, 5µM, Merck) analytical column. Gradient elution was carried out with a mobile phase of 20 mM monobasic sodium phosphate buffer with 0.2% triethylamine (pH 7.0) and acetonitrile at a flow rate of 1.5mL/min. The total run time was 12 min, and the re-equilibration at was 5 min. EMB detection was performed at 210 nm, and RIF, INH and PYZ were detected at 238 nm, using a DAD. The method proved to be specific, linear (r^2>0.99), precise (RSD<2%), accurate, and robust and may be applied to the QC analysis of pharmaceutical formulations[37].

In 2015, *Prasanthi B, et. al.,* developed a rapid, simple, sensitive and cost effective stability indication high performance liquid chromatographic method for the simultaneous determination of rifampicin, isoniazid and pyrazinamide in human plasma. The three drugs were eluted under isocratic mode using a 250X4.0 mm id, 5µm Phenomenex ODS 2 C18 column. The mobile phase was composed of a mixture of acetonitrile, methanol and water in the ration of 30:5:65 (v/v, pH adjusted to 5.2) at flow rate of 1.0mL/min. The limits of detection and quantification for rifampicin were 0.13 and 0.4µg/mL, for isoniazid-0.6 and 1.8µg/mL; and pyrazinamide 0.5 and 1.6µg/mL, respectively. The method can be successfully applied for pharmacokinetic, bioavailability or bioequivalence studies of rifampicin, isoniazid and pyrazinamide combination in human subjects[38].

In 2017, *Hakkimane SS, et. al.,* used isocratic elution with 10 minutes runtime on a C18 Luna, 5I1/4, 100Å , 150 mm column, methanol, and water as mobile phase with detection wavelength at 268 nm. INH nanoformulations were prepared by double emulsion solvent evaporation technique. Quantitative analysis of encapsulated drug was estimated via developed RP-HPLC method. Simultaneous estimation for the two drugs was carried out by gradient elution. All chromatographic separation and estimations were obtained on Shimandzu HPLC system[39].

In 2017, *Mishra P, et. al.,* developed a direct injection liquid chromatographic method for the determination of pyrazinamide in pharmaceutical formulation. The method includes a micellar mobile phase containing 0.15M sodium dodecyl sulphate and 1% butanol (v/v) buffered at pH 3, a Princeton SPHER-100 C18 column (250X4.6mm, 5µm particle size) and UV detection was set at 269nm. The micellar liquid chromatography (MLC) method is rapid, precise, sensitive and robust. In this method pharmaceutical samples were directly injected to the column without pre-treatment step. Under all these conditions, method has very short analysis time of 3.2 min, linearity (r> 0.998), limit of detection and limit of quantification is 1.4, 36.5 ng/ml respectively; intra and inter-day precision (RSD%) were 1.5, overall recovery in pharmaceutical formulation is 99.4%, 69.5%, 81.25%, 87.9%. The method is suitable for routine quality control analysis. This chromatographic techniques, MLC has the advantage of avoiding sample extraction step from matrices, thus reduces the time of analysis[40].

In 2017, *Hermawan D, et. al.,* developed a simple high performance liquid chromatography method for the analysis of miconazole, an antifungal drug in powder sample. The optimized HPLC system using C8 column was achieved using mobile phase composition containing methanol: water (85:15, v/v), a flow rate of 0.8mL/min, and UV detection at 220nm. The calibration graph was linear in the range from 10-50 mg/L with r^2 of 0.9983. The limit of detection (LOD) and limit of quantitation (LOQ) obtained were 2.24mg/L and 7.47mg/L, respectively. The present HPLC method is applicable for the determination of m HPLC method provides short analysis time, high reproducibility and high sensitivity[41].

In 2018, *Khatak S, et. al.,* developed a new simple, rapid and sensitive reversed-phase high-performance liquid chromatography method as per International Conference on Harmonization guidelines, Q2R1, for simultaneous estimation of isoniazid, pyrazinamide and rifampicin in solid lipid nanoparticles. Separation was achieved on a 250X4.6mm, 5µm, C18 column using a linear gradient flow rate of 1.5ml/min. Isoniazid, pyrazinamide and rifampicin were identified based on their retention times as compared to standards and confirmed with characteristic spectra on a spectrophotometer at 238 nm and eluted at 3.787, 4.173 and 11.273 min, respectively, achieved within 20min. This method was linear, precise with %RSD values of 0.18% for isoniazid, 0.15% for pyrazinamide and 0.47% for rifampicin, accurate with mean recovery yields of 101.312% for isoniazid, 99.910% for pyrazinamide and 99.767% for rifampicin and selective over the concentration range of 10-15% for all the three drugs. This method is suitable due to its simplicity and accuracy for routine quality control and stability analysis of Antitubercular drugs-loaded solid lipid nanopaticles[42].

In 2018, *Le Thi Luven TM, et. al.,* described a method that can rapidly and simultaneously measure the plasma concentrations of four anti-tuberculosis drugs (isoniazid, rifampicin, pyrazinamide and ethambutol) and one major metabolite (acetylisoniazid) using high-performance liquid chromatography/tandem mass spectrometry (HPLC/MS/MS). To adjust for degradation and losses during sample preparation, Diltiazem hydrochloride was used as an internal standard. Samples were prepared by using protein precipitation with methanol. Four drugs and one metabolite were simultaneously separated by using the high-performance liquid chromatography system with Gemini C18 column and mobile phase consisting of MeOH and Ammonium Acetate 5mM, pH 3.5. The method was found to have high selectivity. Precision estimated by the coefficient of variation was <15% for all drugs. The linear range of the calibration curves for PZA 1.0-100µg/mL; RIF 0.2-20µg/mL; INH 0.1-10µg/mL; AcINH 0.1-10µg/mL and EMB 20-5000ng/mL. The results of validation for selectivity/specificity, precision, accuracy, linearity, limit of quantification, recovery, and stability show that the method meets the requirements of a bioanalytical method. Our method may serve well for routine therapeutic monitoring of the first-line anti-TB drugs in patient plasma[43].

In 2018, *Luciani-Giacobbe LC, et. al.,* developed a simple and selective reversed phase HPLC-UV method for rifampicin and isoniazid quantification in human plasma. The method consisted of drug extraction with trichloroacetic acid and organic solvent followed by derivatization of isoniazid. Using an isocratic mode, rifampicin was analyzed on a C18 (250X4.6mm, 5µm) column at 339 nm, while isoniazid was analyzed on a C8 (250X4.6mm, 5µm) column at 273 nm. All validation parameters fulfilled the FDA requirements, as the method was accurate, precise and linear from 0.31 to 37.80 µg/mL of isoniazid. The samples remained stable during the usual processing and analysis times and also during the two freeze/thaw cycles. The recovery of both analytes was reproducible in the range of 97.3-99.6% of rifampicin and 89.8-96.6% of isoniazid. The low volume of plasma necessary for the quantification of the samples (750µL in total) and the low limit of quantification (0.31µg/mL for rifampicin and 0.89µg/mL for isoniazid) made this method useful for carrying out pharmacokinetic tests both in humans or animal models. In addition, the method can be successfully applied for bioavailability studies or drug monitoring in tuberculosis treatment[44].

In 2018, *Gao S, et. al.,* developed a simple and sensitive method based on liquid chromatography-tandem mass spectrometry (LC-MS/MS) and single protein precipitation for simultaneously quantifying of pyrazinamide, isoniazid, ethambutol, streptomycin and rifampicin in human plasma. Optimized chromatographic separation was achieved on a ZORBX SB-C18 column with heptafluorobutyric acid, an ion-pair reagent, in the mobile phase at a flow rate of 0.3mL/min. The mass detection was achieved using electrospray ionization in the positive ion mode with multiple reactions monitoring mode. The lower limit of quantification (LLOQ) and dynamic range of pyrazinamide, isoniazid, ethambutol, streptomycin and rifampicin were 200-4000 ng/mL, 80-2000ng/mL, 0.2-1000 ng/mL, 2000-200000 ng/mL and 200-4000 ng/mL, respectively. The inter-day and intra-day accuracy and precision were within ± 15.0% and less than 15%. The

method had been successfully applied to simultaneous determination of four first-line Anti-tuberculosis drugs in plasma from tuberculosis patients[45].

In 2018, *Mishra P, et. al.*, described the development of a micellar liquid chromatographic method to quantify isoniazid in urine samples. Extraction steps were avoided, making the procedure easy to handle and reducing the waste of toxic organic solvents. Isoniazid was eluted in less than 5 min without interference from other compounds of the urine using a mobile phase containing 0.15 SDS-12.5% 1-propanol (v/v)-Na_2HPO_4 0.01 M buffered at pH 7, running at 1mL/min under isocratic mode through a C18 column with the detection wavelength at 265 nm. The method was validated by following the requirements of the guidelines on Bioanalytical Method Validation issued by the European Medicines Agency (EMA) in terms of selectivity, calibration curve (r^2 = 0.9998 in the calibration range 0.03-10.0 µg/mL), limit of detection and quantification (10 and 30 ng/mL respectively), precision (<16.0%), accuracy (-0.9 to + 8.5%), carry-over, matrix effect, and robustness. The developed method was applied to quantify isoniazid in urine samples of patients of an India hospital with good results. The method was found to be useful for routine analysis to check the amount of isoniazid in these patients and could be used its therapeutic monitoring[46].

In 2018, *Laila L, et.al.*, developed a selective, reproducibility, effective, sensitive, simple and fast High-Performance Liquid Chromatography (HPLC) to analyze 25-Desacetyl Rifampicin (25-DR) in human urine which is from tuberculosis patient. The separation was performed by HPLC Agilent Technologies with column Agilent Eclipse XDB-C18 and a mobile phase of 65:35 v/v methanol: 0.01M sodium phosphate buffer pH 5.2, at 254 nm and flow rate of 0.8ml/min. The mean retention time was 3.016 minutes. The method was linear from 2-10µg/ml 25-DR with a correlation coefficient of 0.9978. Standard deviation, relative standard deviation and coefficient variation of 2,6,10 µg/ml 25-DR were 0-0.0829, 03.1752, 0-0.0317%, respectively. The recovery of 5, 7, 9 µg/ml 25-DR was 80.8661, 91.3480 and 111.1457%, respectively. Limit of detection (LOD) and quantification (LOQ) were 0.51 and 1.7µg/ml, respectively. The method has fulfilled the validity guidelines of the International Conference on Harmonization (ICH) bioanalytical method which includes parameters of specificity, linearity, precision, accuracy, LOD, LOQ. The developed method is suitable for pharmacokinetic analysis of various concentrations of 25-DR in human urine[47].

In 2018, *Khatak S, et. al.*, developed a rapid, simple, sensitive and cost effective stability indicating high performance liquid chromatographic method for the simultaneous determination of rifampicin, isoniazid, and pyrazinamide in human plasma. The three drugs were eluted under isocratic mode using a 250X4.0 mm i.d., 5µm Phenomenex ODS 2 C18 column. The mobile phase was composed of a mixture of acetonitrile, methanol and water in the ration of 30:5:65 (v/v, pH adjusted to 5.2) at a flow rate of 1.0mL/min. The limit of detection and quantification for rifampicin were 0.13 and 0.4µg/mL, for isoniazid-0.6 and 1.8µg/mL; and for pyrazinamide-0.5 and 1.6µg/mL, respectively. The method can be successfully applied for pharmacokinetic, bioavailability or bioequivalence studies of rifampicin, isoniazid and pyrazinamide combination in human subjects[48].

In 2019, *Shah P, et. al.*, developed a accurate, precise and robust isocratic HPLC method for simultaneous determination of Rifampicin and Ofloxacin. The chromatographic separation was carried out on Kinetex C18, 100 A Phenomenex column with a mixture of 0.03M Potassium dihydrogen phosphate buffer pH 3.0: Acetonitrile (55:45) as mobile phase at 230 nm. The retention times were 2.91 and 4.87 min for Ofloxacin and Rifampicin, respectively. Calibration plots were linear over the concentration range 1-5 and 2-10µg/mL for Rifampicin and Ofloxacin, respectively. The method was validated for linearity, sensitivity accuracy, precision, and robustness. Percent recoveries were found to be close to 100% with low variability. Fractional factorial design with four factors was chosen for robustness testing. The volume of acetonitrile and flow rate showed significant effect on retention factor of both the drugs and asymmetry factor of ofloxacin. The method may be adopted for routine analysis at industry[49].

In 2019, *Temova Rakusa Z, et. al.*, developed a simple, fast and cost-effective LC-MS/MS method for quantification of rifampicin in human plasma. The plasma samples containing rifampicin and isotopically labelled internal standard rifampicin D8, were cleaned up using a Captiva ND lipids filtration plate. Chromatograph separation was achieved on an 1290 Infinity liquid chromatography coupled to 6460 Triple Quadrupole operated in positive mode on a core-shell Kinetex C18 column (50 X 2.1mm, 2.6µm) by gradient elution using 0.1% formic acid in water and acetonitrile as a mobile phase. The proposed method is the fastest method published by now, both in terms of sample preparation and chromatographic analysis (total run time 2.4min). Another key benefit is the outstanding sensitivity and wide analytical range (5-40000µg/L) with good

linearity, accuracy and precision. The method showed almost complete recovery (92%) and absence of any significant matrix effect as demonstrated by uniform responses from QC samples prepared in blood plasma from 6 volunteers. The proposed method was successfully applied to rifampicin quantification in 340 patient's plasma samples, thus demonstrating its suitability for both therapeutic drug monitoring and pharmacokinetic analysis[50].

Conclusion:

This review focuses on introductory aspects of tuberculosis, symptoms, some newer analogues under investigation and various analytical, bioanalytical methods reported on antitubercular agents. This paper will definitely help graduate researcher working in analytical chemistry to define their direction in liquid chromatographic method development.

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