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# REVIEW: AVAILABLE ANALYTICAL METHODS FOR THE ESTIMATION OF FIRST LINE, SECOND LINE ORAL AND NEWER ANTI-TB DRUGS

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**Keywords:**

Bedaquiline, Delamanid, Pretomanid, RP-HPLC, HPTLC, LC-MS

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**ABSTRACT:** Tuberculosis (TB) is one of the top ten causes of death worldwide. Presently, one-quarter of the world's population is thought to be infected with TB. New infections occur in about 1% of the population each year. Tuberculosis is a major global health threat. There is a progressive increase in multidrug-resistant (MDR) and extensively drug-resistant tuberculosis (XDR). Multi drug resistance (MDR)-TB and Extensively drug resistance (XDR)-TB poses a vital challenge to the control of tuberculosis. Numbers of drugs are available in the market for the treatments of tuberculosis as well as many new drugs are also available for the treatment of MDR-TB and XDR-TB. This review article covers most of the different official and reported analytical methods for the estimation of the first line, second line oral, and newer anti-TB drugs. The main objective of this review is to classify, summarize, and discusses the different proposed analytical methods for the estimation of above mentioned anti-TB drugs alone and in combination with other drugs in bulk, pharmaceutical formulation and biological matrices.

**INTRODUCTION:** Tuberculosis (TB) is the most important airborne infectious disease caused by a bacterium called Mycobacterium tuberculosis (MTB). The one third of the world population is the infected by mycobacterium tuberculosis according to the World Health Organisation (WHO) estimation. HIV infected persons, immigrants from countries with high rates of tuberculosis, the homeless, healthcare professionals, intravenous drug users, a person taking immunosuppressive agents and those in an institutional setting such as nursing homes and correctional facilities group at high risk for tuberculosis infection there is a progressive increase in multidrug-resistant (MDR) and extensively drug-resistant tuberculosis (XDR).

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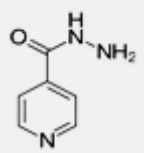
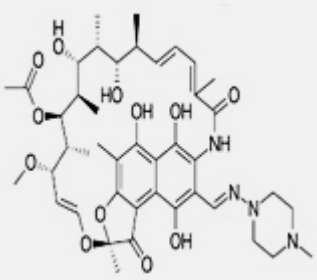
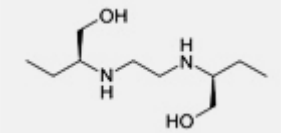
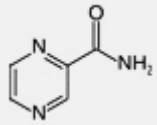
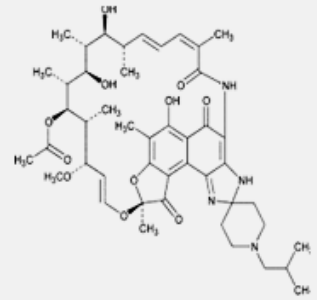
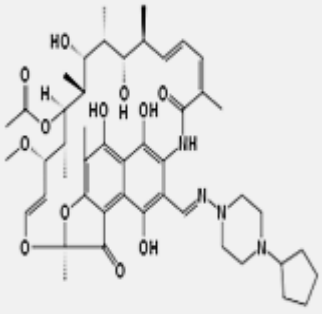
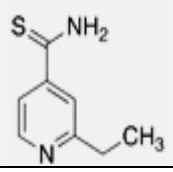
Anti-Tb drugs are classified as:

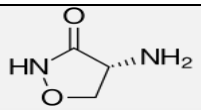
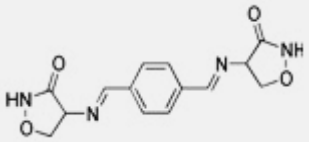
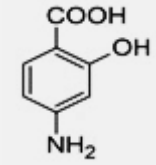
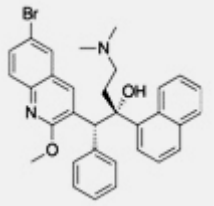
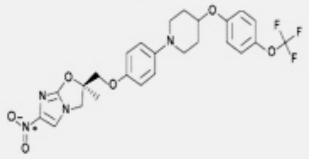
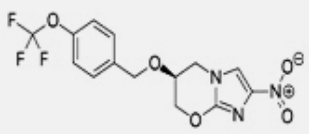
(i) Oral first-line drugs and extended first-line drugs (Isoniazid, Rifampicin, Ethambutol, Pyrazin-amide, Rifabutin, Rifapentine), (ii) Injectable anti-TB drugs (Streptomycin, Kanamycin, Amikacin, Capreomycin, Viomycin) (iii) Fluoroquinolones (Ciprofloxacin, Ofloxacin, Levofloxacin, Moxi-floxacin, Gatifloxacin) (iv) Oral second-line anti-TB drugs (Ethionamide/Prothionamide, Cycloserine, Terizi-done, Para-aminosalicylic acid) (v) Anti-TB drugs with limited data on efficacy and long-term safety in the treatment of drug-resistant TB (This group includes new anti-TB agents) (Bedaquiline, Delamanid, Pretomanid, Linezolid, Clofazimine, Amoxicillin / clavulanate, Imipenem / cilastatin, Meropenem, High-dose Isoniazid, Thioacetazone, Clarithromycin).

The increasing interest in the oral first line and the oral second line as well as new anti-TB drugs like bedaquiline, delamanid, and pretomanid led us to review the official and reported analytical methods

for the estimation of these anti-TB drugs alone and in combination with other drugs in bulk, pharmaceutical formulation and biological matrices.

**TABLE 1: DRUG PROFILE OF ANTI-TB DRUGS (ORAL FIRST LINE, ORAL SECOND LINE AND NEWER ANTI-TB DRUGS)**

| Drug | Chemical Structure | Chemical Name | Chemical Formula | pKa | Log P |
|---|---|---|---|---|---|
| Isoniazid |  | 4-Pyridine-carboxylic acid hydrazide | $C_6H_7N_3O$ | 1.82 | -0.8 |
| Rifampicin |  | (2S,12Z,14E,16S,17S,18R,19R,20R, 21S,22R,23S,24E)-1,2-dihydro-5,6,9,17,19-pentahydroxy-23-methoxy-2,4,12,16,18,20,22-heptamethyl-8-(4-methylpiperazin-1-yliminomethyl)-1,11-dioxo-2,7-(epoxypentadeca-1,11,13-trienoimino)naphtho[2,1-b]furan-21-yl acetate | $C_{43}H_{58}N_4O_{12}$ | 1.7 | 2.7 |
| Ethambutol |  | 2,2'-(1,2-Ethanediyldiimino)-bis-1-butanol | $C_{10}H_{24}N_2O_2$ | 9.49 | -0.3 |
| Pyrazinamide |  | Pyrazinecarboxamide or Pyrazine-2-carboxamide | $C_5H_5N_3O$ | 0.5 | -0.6 |
| Rifabutin |  | 9S,12E,14S,15R,16S,17R,18R,19R, 20S,21S,22E,24Z)-6,16,18,20-Tetrahydroxy-1'-isobutyl-14-methoxy-7,9,15,17,19,21,25-heptamethylspiro[9,4-(epoxypentadeca[1,11,13]trienimino)-2H-furo[2',3':7,8]naphth[1,2-d]imidazole-2,4'-piperidine]-5,10,26-(3H,9H)-trione-16-acetate | $C_{46}H_{62}N_4O_{11}$ | 6.9 | 4.5 |
| Rifapentine |  | (2S,12Z,14E,16S,17S,18R,19R,20R, 21S,22R,23S,24E)-8-{(E)-[(4-cyclopentylpiperazin-1-yl)imino]methyl}-5,6,9,17,19-pentahydroxy-23-methoxy-2,4,12,16,18,20,22-heptamethyl-1,11-dioxo-1,2-dihydro-2,7-(epoxypentadeca[1,11,13]trienimino)naphtho[2,1-b]furan-21-yl acetate | $C_{47}H_{64}N_4O_{12}$ | -1.6 | 4 |
| Ethionamide |  | 2-Ethyl-4-pyridinecarbothioamide | $C_8H_{10}N_2S$ | 4.49 | 0.5 |

| | | | | | |
|---|---|---|---|---|---|
| Cycloserine |  | D-4-Amino-3-isoxazolidinone | $C_3H_6N_2O_2$ | 4.4, 7.4 | -0.9 |
| Terizidone |  | 4-[({4-[N-(3-oxo-1,2-oxazolidin-4-yl)carboximidoyl]phenyl}methylidene)amino]-1,2oxazolidin-3-one | $C_{14}H_{14}N_4O_4$ | 3.54 | 0.17 |
| p-aminosalicylic acid |  | 4-Aminosalicylic acid or 4-Amino-2-hydroxybenzoic acid | $C_7H_7NO_3$ | 3.25 | 1.6 |
| Bedaquiline |  | (1R,2S)-1-(6-bromo-2-methoxyquinolin-3-yl)-4-(dimethylamino)-2-naphthalen-1-yl-1-phenylbutan-2-ol | $C_{32}H_{31}BrN_2O_2$ | 1.57 | 7.25 |
| Delamanid |  | (2R)-2-methyl-6-nitro-2-[(4-{4-[4-(trifluoromethoxy)phenoxy]-1-piperidinyl}phenoxy)methyl]-2,3-dihydroimidazo[2,1-b][1,3]oxazole | $C_{25}H_{25}F_3N_4O_6$ | 5.51 | 6.14 |
| Pretomanid |  | 6S)-2-nitro-6-{[4-(trifluoromethoxy)phenyl]methoxy}-6,7-dihydro-5H-imidazo[2,1-b][1,3]oxazine | $C_{14}H_{12}F_3N_3O_5$ | -3 | 4.14 |

**Analytical Methods for Anti-TB Drugs:** Different official and reported analytical methods such as UV-visible spectrophotometric, Spectro-fluorometric, High-performance liquid chromato-graphy (HPLC), High-performance thin-layer chromatography (HPTLC), Gas chromatography (GC), Micellar electro-kinetic capillary chromato-graphy, Electrochemical, Titrimetric, Liquid chromatography / Mass spectrometry (LC/MS), Capillary-electrophoresis, Flow injection analysis, Chemi-luminescence, *etc*. are available for estimation of the first line, oral second line and newer anti-TB drugs in bulk, pharmaceutical formulation and biological matrices.

A review of different official and reported analytical methods is listed as follow:

## Official Analytical Methods:

**TABLE 2: UV-VISIBLE SPECTROPHOTOMETRIC METHODS**

| S. no. | Drug / Sample | Pharmacopoeia | Solvent | Wavelength of Detection | Ref. no. |
|---|---|---|---|---|---|
| 1 | Cycloserine Tablet | IP 2018 | Water, 0.2 M NaOH, 1 M acetic acid, sodium nitroprusside solution | 625 nm | 29 |
| 2 | Ethionamide & Ethionamide Tablets | USP 2013 | Methanol | 290 nm | 30 |
| 3 | Rifampicin, Rifampicin Capsule & Rifampicin Oral Suspension | BP 2016 | Methanol, phosphates buffer pH 7.4 | 475 nm | 31 |
| 4 | Pyrazinamide Tablets | IP 2018<br>BP 2016 | Water | 268 nm | 49<br>32 |
| 5 | Rifampicin | EP 2008 | Methanol, phosphates buffer pH 7.4 | 475 nm | 33 |

**TABLE 3: LIQUID CHROMATOGRAPHIC METHODS**

| S. no. | Drug / Sample | Pharmacopoeia | Column | Mobile phase | Flow Rate (ml/ min) | Wavelength of Detection | Ref. no. |
|---|---|---|---|---|---|---|---|
| 1 | Isoniazid & Isoniazid Tablets | IP 2018 | ODS (15 cm × 4.6 mm, 5 µm) | A mixture of a solution prepared by dissolving 1.4 g disodium hydrogen phosphate and 1 ml of triethylamine to 1000 ml with water (pH 6.0 adjusted by OPA) & Acetonitrile (96:4, v/v) | 1 | 265 nm | 34 |
| 2 | Rifampicin, Rifampicin Capsules, Rifampicin Oral Suspension &Rifampicin Tablets | IP 2018 | Octylsilane (10 cm × 4.6 mm, 5 µm) | A mixture of a solution containing 0.1% v/v of OPA, 0.19% w/v of sodium perchlorate, 0.59% w/v of citric acid &2.09% w/v of potassium dihydrogen phosphate & Acetonitrile (65:35, v/v) | 1.5 | 254 nm | 35 |
| 3 | Rifampicin and Isoniazid Tablets | IP 2018 | ODS (25 cm × 4.6 mm, 5 µm) | A. A mixture of a buffer solution pH 6.8 prepared by dissolving 1.4 g disodium hydrogen orthophosphate anhydrous in 1000 ml of water (pH 6.8 ± 0.05 adjusted by dil. phosphoric acid) & Acetonitrile (96:4, v/v)<br>B. A mixture of the buffer solution and Acetonitrile (45:55, v/v) Using mixture, A & B ingradient programme | 1.5 | 238 nm | 35 |
| 4 | Rifampicin, Isoniazid and Ethambutol Tablets<br>1) For Rifampicin and Isoniazid Tablets<br>2) For Ethambutol Hydrochloride | IP 2018 | 1. ODS (25 cm × 4.6 mm, 5 µm)<br>2. Zorbax SB CN (15 cm × 4.6 mm, 5 µm) | 1) A. A mixture of a buffer solution pH 6.8 prepared by dissolving 1.4 g disodium hydrogen orthophosphate anhydrous in 1000 ml of water (pH 6.8 ± 0.05 adjusted by dil. phosphoric acid) & Acetonitrile (96: 4, v/v)<br>B. A mixture of the buffer solution and Acetonitrile (45:55, v/v)<br>Using mixture, A & B in gradient elution programme<br>2) A mixture of Acetonitrile & buffer solution pH 7.0 prepared by dissolving 1 ml of triethylamine in 1000 ml of water (pH 7.0 adjusted by dil. phosphoric acid (50:50, v/v) | 1) 1.5<br>2) 1 | 1) 238 nm<br>2) 200 nm | 35 |
| 5 | Rifampicin, Isoniazid and Pyrazinamide Tablets | IP 2018 | ODS (25 cm × 4.6 mm, 5 µm) | A. A mixture of a buffer solution pH 6.8 prepared by dissolving 1.4 g disodium hydrogen orthophosphate anhydrous in 1000 ml of water (pH 6.8 ± 0.05 adjusted by dil. phosphoric acid) & Acetonitrile (96: 4, v/v)<br>B. A mixture of the buffer solution and Acetonitrile (45:55, v/v)<br>Using mixture, A & B in | 1.5 | 238 nm | 35 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | | gradient programme | | | |
| 6 | Rifampicin, Isoniazid, Pyrazinamide and Ethambutol Tablets<br>For Rifampicin Isoniazid and pyrazinamide Tablets<br>2) For Ethambutol Hydrochloride | IP 2018 | ODS (25 cm × 4.6 mm, 5 µm)<br><br>Zorbax SB CN (15 cm × 4.6 mm, 5 µm) | 1) A. A mixture of a buffer solution pH 6.8 prepared by dissolving 1.4 g disodium hydrogen orthophosphate anhydrous in 1000 ml of water (pH 6.8 ± 0.05 adjusted by dil. phosphoric acid) & Acetonitrile (96: 4, v/v)<br>B. A mixture of the buffer solution and Acetonitrile (45:55, v/v)<br>Using mixture, A & B in gradient programme<br>2) A mixture of Acetonitrile & buffer solution pH 7.0 prepared by dissolving 1 ml of triethylamine in 1000 ml of water (pH 7.0 adjusted by dil. Phosphoric acid) (50:50, v/v) | 1) 1.5<br>2) 1 | 1) 238 nm<br>2) 200 nm | 35 |
| 7 | Ethambutol hydrochloride, Ethambutol Injection & Ethambutol Tablets | IP 2018 | Zorbax SB CN (15 cm × 4.6 mm, 5 µm) | A mixture of buffer solution prepared by dissolving 1 ml of triethylamine in 1000 ml of water (pH 7.0 adjusted by orthophosphoric acid) & Acetonitrile (50:50, v/v) | 1 | 200 nm | 36 |
| 8 | Ethambutol & Isoniazid Tablets<br>1) For Isoniazid<br>2) For Ethambutol | IP 2018 | 1) ODS (15 cm × 4.6 mm, 5 µm)<br>2) Zorbax SB CN (15 cm × 4.6 mm, 5 µm) | 1) A mixture of buffer solution pH 6.8 prepared by dissolving 1.4 g disodium hydrogen phosphate in 1000 ml of water (pH 6.8 ± 0.05 adjusted by dil. phosphoric acid) & Acetonitrile (96:4, v/v)<br>2) A mixture of buffer solution prepared by dissolving 1 ml of triethylamine in 1000 ml of water (pH 7.0 ± 0.05 adjusted by phosphoric acid) & Acetonitrile (50:50, v/v) | 1 | 1) 254 nm<br>2) 200 nm | 36 |
| 9 | Ethionamide & Ethionamide Tablets | IP 2018 | ODS (25 cm × 4.6 mm, 5 µm) | A mixture of buffer solution prepared by dissolving 2 ml of triethylamine in 1000 ml of water (pH 6.0 adjusted by orthophosphoric acid) & Acetonitrile (60:40, v/v) | 1 | 290 nm | 37 |
| 10 | Cycloserine & Cycloserine Capsules | IP 2018 | Octylsilane (25 cm × 4.6 mm, 5 µm) | 0.1% w/v of methane sulphonic acid & 0.78% w/v potassium dihydrogen orthophosphate in water (pH 6.0 adjusted by dil. NaOH) | 1 | 227 nm | 29 |
| 11 | Isoniazid Injection & Isoniazid Tablets | JP 2006 | ODS (15 cm × 4.6 mm, 5 µm) | Dissolve 6.80 g of potassium dihydrogen phosphate in water to make 1000 ml. Separately, to 5.76 g of phosphoric acid add water to make 1000 ml. Mix these solutions to make a solution having pH 2.5. To 500/400 ml of this solution, add 500/600 ml methanol & add 2.86g of sodium | - | 265 nm | 38 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | | tridecanesulfonate to dissolve. | | | |
| 12 | Rifampicin & Rifampicin Capsules | JP 2006 | ODS (10 cm × 4.6 mm, 5 µm) | Dissolve 4.2 g of citric acid monohydrate and 1.4 g of sodium perchlorate in 1000 ml of a mixture of water, acetonitrile and phosphate buffer solution, pH 3.1 (11:7:2, v/v/v) | - | 254 nm | 39 |
| 13 | Isoniazid & Isoniazid Injection | USP 2013 | ODS (25 cm × 4.6 mm, 1.5 to 10 µm) | Dissolve 4.4. g of docusate sodium in 600 ml of methanol, add 400 ml of water, (pH 2.5 adjusted by 2N sulfuric acid) | 1.5 | 254 nm | 40 |
| 14 | Isoniazid Tablets | USP 2013 | ODS (30 cm × 3.9 mm, 1.5 to 10 µm) | Buffer: Methanol (95:5, v/v)<br>Buffer solution: Prepare a 0.1 M monobasic potassium phosphate solution, adjust with 10 N NaOH to pH of 6.9, add sufficient triethanolamine to obtain a solution having a known concentration of 0.2 mM of triethanolamine & mix | 1.5 | 254 nm | 40 |
| 15 | Rifabutin, Rifabutin Capsules & Rifabutin Oral Suspension | USP 2013 | Octylsilane (12.5 cm × 4.6 mm, 5 µm)<br>-For Rifabutin oral suspension Octylsilane (15 cm × 4.6 mm, 5 µm) | A mixture of acetonitrile & 0.1 M monobasic potassium phosphate (pH 6.5 ± 0.1 adjusted by 2N NaOH) (50:50, v/v) | 1 | 254 nm | 41 |
| 16 | Rifampicin, Rifampicin Capsules, Rifampicin for Injection & Rifampicin Oral Suspension | USP 2013 | Octylsilane (10 cm × 4.6 mm, 5 µm) | A mixture of water, acetonitrile, phosphate buffer, 0.1 M citric acid & 0.5 M sodium perchlorate (510:350:100:20:20, v/v/v/v/v)<br>For Rifampicin Oral Suspension: (500:360:100:20:20, v/v/v/v/v) | 1.5 | 254 nm | 42 |
| 17 | Rifampicin and Isoniazid Capsules, Rifampicin Isoniazid and Pyrazinamide Tablets, Rifampicin, Isoniazid, Pyrazinamide & Ethambutol Hydrochloride Tablets | USP 2013 | ODS (25 cm × 4.6 mm, 5 µm)<br>-For Ethambutol HCl:<br>CN (15 cm × 4.6 mm, 5 µm) | Buffer solution- Dissolve 1.4 g of dibasic sodium phosphate in 1L of water (pH 6.8 adjusted by phosphoric acid)<br>Solution A: Buffer solution & Acetonitrile (96:4, v/v)<br>Solution B: Buffer solution & Acetonitrile (45:55, v/v)<br>Use variable mixtures of solution A & B in gradient elution programme<br>- For Ethambutol Hydrochloride:<br>Buffer solution: Mix 1.0 ml of triethylamine and 1 L of water (pH 7 adjusted by phosphoric acid)<br>Use mixture of Acetonitrile & Buffer solution (50:50, v/v) | 1.5<br>1 | 238 nm<br>200 nm | 42 |
| 18 | Pyrazinamide Oral suspension | USP 2013 | Octylsilane (25 cm × 4.6 mm, 5 µm) | Acetonitrile and solution of 10 mM monobasic sodium phosphate (pH 3.5 adjusted | 0.8 | 215 nm | 43 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | | by phosphoric acid) (10:90, v/v) | | | |
| 19 | Pyrazinamide Tablets | USP 2013 | ODS (15 cm × 3.9 mm, 1.5 to 10 µm) | Prepare pH 8.0 phosphate buffer (pH 3.0 adjusted by phosphoric acid). Mix 10 ml of acetonitrile with 1 L of this solution. | 1 | 270 nm | 43 |
| 20 | Ethambutol Hydrochloride Tablets | USP 2013 | CN (15 cm × 4.6 mm, 5 µm) | Acetonitrile & Buffer solution prepared by mixing 1.0 ml of triethylamine and 1 L of water (pH 7 adjusted by phosphoric acid) (1:1, v/v) | 1 | 200 nm | 44 |
| 21 | Amino Salicylic Acid & Amino Salicylic Acid Tablets | USP 2013 | ODS (25 cm × 4.6 mm, 1.5 to 10 µm) | Mixture of 0.05 M dibasic sodium phosphate, 0.05 m monobasic sodium phosphate & methanol containing 1.9 g of tetrabutyl-ammonium hydroxide (425:425:150, v/v/v) | 1.5 | 254 nm | 45 |
| 22 | Cycloserine & Cycloserine Capsules | USP 2013 | ODS (25 cm × 4.6 mm, 5 µm) | Dissolve 0.5 g of sodium 1-decanesulfonate in 800 ml water, add 50 ml of acetonitrile & 5 ml of glacial acetic acid (pH 4.4 adjusted by 1 N NaOH) | 1 | 219 nm | 46 |
| 23 | Rifabutin | BP 2016 | Octylsilyl (0.110 m × 4.6 mm, 5 µm) | Acetonitrile & a 13.6 g/ml solution of potassium dihydrogen phosphate (pH 6.5 adjusted by dil. NaOH) | 1 | 254 nm | 47 |
| 24 | Rifabutin | EP 2008 | Octylsilyl (0.110 m × 4.6 mm, 5 µm) | Acetonitrile & a 13.6 g/ml solution of potassium dihydrogen phosphate (pH 6.5 adjusted by dil. NaOH) | 1 | 254 nm | 48 |

**TABLE 4: TITERIMETRIC METHOD**

| S. no. | Drug / Sample | Pharmacopoeia | Description | Ref. no. |
|---|---|---|---|---|
| 1 | Pyrazinamide | IP 2018 | Weigh 0.3 g Pyrazinamide & transfer to the flask of ammonium distillation apparatus. Add 200 ml of water & 75 ml NaOH solution. Boil & collecting the distillate in 50 ml of 0.05 M sulphuric acid. Boil to the complete distillation of the ammonia and titrate the excess of acid with 0.1 M NaOH, using methyl red as an indicator. | 49 |
| 2 | Isoniazid Oral Solution | USP 2013 | Isoniazid Oral Solution in 50 ml of a mixture of 1 part of KBr in 10 parts of dil. HCl. Proceed as per Nitrite Titration. | 40 |
| 3 | Pyrazinamide | USP 2013 | Place about 300 mg of Pyrazinamide in 500 ml Kjeldahl flask, dissolve in 100 ml of water & add 75 ml of 5 N NaOH. Connect the flask to the condenser, the delivery tube of which dips into 20 ml of boric acid solution. Boil vigorously to complete the distillation of the ammonia. After cooling, add methyl purple & titrate with 0.1 N HCl. | 43 |
| 4 | Ethambutol Hydrochloride | USP 2013 | Add 200 mg of Ethambutol Hydrochloride in a mixture of 100 ml of glacial acetic acid & 5 ml of mercuric acetate. Add crystal violet. Titrate with 0.1N perchloric acid. The color change at the endpoint is from blue to blue-green. | 44 |
| 5 | Isoniazid | BP 2016<br>EP 2008 | Dissolve 0.250 g Isoniazid in water and dilute 100 ml with water. To 20 ml of the solution, add 100 ml of water, 20 ml HCl, 0.2 g of KBr, and 0.05 ml methyl red solution. Titrate with 0.0167 M potassium bromate until the red color disappears. | 50<br>51 |
| 6 | Isoniazid Injection | BP 2016 | Dilute 0.4 g of Isoniazid to 250 ml with water. To 25 ml of the solution, add 25 ml of 0.05 M Br & 5 ml HCl. Allow standing for 15 min. Add 1 g of KI & titrate with 0.1 M sodium thiosulphate using starch mucilage as an indicator. | 50 |
| 7 | Isoniazid Tablets | BP 2016 | Dissolve a quantity equivalent to 0.4 g of Isoniazid and dilute 100 ml with water. To 50 ml of the solution, add 50 ml of water, 20 ml HCl, 0.2 g of KBr, and titrate with 0.0167 M potassium bromate. Determining the end-point electrometrically. | 50 |
| 8 | Pyrazinamide | BP 2016 | Dissolve 0.100 g Pyrazinamide in 50 ml of acetic anhydride. Titrate with | 32 |

| | | | | |
|---|---|---|---|---|
| | | EP 2008 | 0.1 M perchloric acid, determining the end point potentiometrically. | 52 |
| 9 | Ethambutol Hydrochloride | BP 2016<br>EP 2008 | Dissolve 0.200 g of Ethambutol Hydrochloride in 50 ml of water & add 1 ml of 0.1 M HCl. Carry out a potentiometric titration, using 0.1 M NaOH. | 53<br>54 |
| 10 | Ethambutol Tablets | BP 2016 | Add 20 ml of 2 M NaOH to a quantity of tablet powder equivalent to 0.2 g of Ethambutol Hydrochloride. Extract with three successive 25 ml quantities of a mixture of 3 volumes of chloroform and 1 volume of propan-2-ol. Filter each extract. Add 100 ml anhydrous acetic acid to the combined extracts & carry out non-aqueous titration, using 1-naphtholbenzein solution as an indicator. | 53 |
| 11 | Ethionamide | BP 2016<br>EP 2008 | Dissolve 0.150 g of Ethionamide in 50 ml anhydrous acetic acid. Titrate with 0.1 M perchloric acid, determining the end point potentiometrically. | 55<br>56 |
| 12 | Isoniazid | JP 2006 | Dissolve 0.3 g Isoniazid in 50 ml acetic acid & 10 ml of acetic anhydride. Titrate with 0.1 mol/L perchloric acids, until the color of the solution changes from yellow to green. | 38 |
| 13 | Pyrazinamide | JP 2006 | Dissolve 0.1 g Pyrazinamide in 50 ml of acetic anhydride. Titrate with 0.1 mol/L perchloric acid, determining the end point potentiometrically. | 57 |
| 14 | Ethambutol Hydrochloride | JP 2006 | Dissolve 0.2 g Ethambutol Hydrochloride in 20 ml of water & add 1.8 ml copper (II) sulfate. Add 7 ml of NaOH with shaking, add water to make 50 ml. To 10 ml of this solution, add 10 ml of ammonia-ammonium chloride buffer of pH 10.0 and 100 ml water. Titrate with 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate until the color of the solution changes from blue-purple to light yellow. | 58 |
| 15 | Ethionamide | JP 2006 | Dissolve 0.3 g Ethionamide in 50 ml of acetic acid. Titrate with 0.1 mol/L perchloric acids, until the color of the solution changes from orange-red to dark orange-brown, using 2 ml p-naphtholbenzein solution as an indicator. | 59 |

## Reported Analytical Methods:

## 1. UV-Visible Spectrophotometric Methods:

**TABLE 5: UV-VISIBLE SPECTROPHOTOMETRIC METHODS**

| S. no. | Drug / Sample | Method | Solvent | Linearity Range µg/ml | Wavelength of Detection | Ref. no. |
|---|---|---|---|---|---|---|
| | | **Isoniazid** | | | | |
| 1 | Isoniazid in urine | Spectrophotometric (Based on the formation of an orange, yellow colour complex between isoniazid and ammonium metavanadate in an acid medium) | Distilled water | 1.37-13.70 | 420 nm | 60 |
| 2 | Isoniazid in pharmaceuticals | Spectrophotometric (Using Cerium (IV) and Two Acid Dyes) Method A (using methyl orange) Method B (using indigo carmine) | Distilled water | For Method A: 0.3–3.0 For Method B: 0.5–7.0 | For Method A: 520 nm For Method B: 610 nm | 61 |
| 3 | Isoniazid in presence of Rifampicin in pharmaceuticals & urine | Spectrophotometric (using isatin as a reagent) | Distilled water | 0-32 | 340 nm | 62 |
| 4 | Isoniazid in pure & pharmaceutical formulation | Spectrophotometric (Based on the oxidation of 4,5-dihydroxy-1,3-benzenedisulfonic acid (Tiron) by sodium metaperiodate (SPI) followed by oxidative coupling with INH in an alkaline medium. | Distilled water | 1-15 | 507 nm | 63 |
| 5 | Isoniazid in pharmaceuticals | Spectrophotometric (Redox-Reaction Based) Method A (using Folin-Ciocalteu reagent) Method B (using iron (III) and ferricyanide) | Water | For Method A: 0.5–10.0 For Method B: 0.2–3.0 | 760 nm | 64 |
| 6 | Isoniazid in pharmaceuticals | Spectrophotometric (Using 6,7-dichloroquinoline-5,8-dione) | Water & Ethanol | 2–25 | 645 nm | 65 |
| 7 | Isoniazid in pharmaceuticals | Spectrophotometric (Using Natural Aldehyde like cis- | Distilled Water | 0.5-2.5 | 364 nm | 66 |

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| | | cinnamaldehyde) | | | | |
| 8 | Isoniazid in pharmaceuticals | Spectrophotometric (Using its Schiff's base derivatives) | Methanol | 0.25-5 | 421 nm | 67 |
| 9 | Isoniazid in bulk & pharmaceuticals | Visible Spectrophotometric (based on the formation of yellow colored chromogen with ethanolic p-dimethylamino benzaldehyde solution in the presence of conc. HCl) | Ethanol | 100-600 | 395 nm | 68 |
| 10 | Isoniazid in pure & pharmaceutical formulation | Spectrophotometric (Using vanillin) | 0.5M ethanolic HCl acid | 1-12 | 405 nm | 69 |
| 11 | Isoniazid in Tablets | Colorimetric | Distilled water | 3-18 | 530 nm | 70 |
| 12 | Isoniazid in bulk & pharmaceutical dosage forms. | Colorimetric (using ethyl vanillin in presence of 0.5M NaOH) | Distilled Water | 2-16 | 410 nm | 71 |
| | | Isoniazid in combination with other drugs | | | | |
| 13 | Isoniazid and Pyridoxine in tablet dosage form | Spectrophotometric (Simultaneous Equation Method) | Distilled Water | 5-25 | Isoniazid: 263 nm Pyridoxine: 290 nm | 72 |
| 14 | Isoniazid (INH) and Ritodrine Hydrochloride (RTH) in pure dosage forms | Spectrophotometric (Based on the diazotisation of 4,4'-sulphonyldianiline (dapsone, DAP) followed by a coupling reaction with either INH or RTH in sodium hydroxide medium) | Deionised water | Isoniazid: 0.5-20 Ritodrine HCl: 0.5-18 | Isoniazid: 440 nm Ritodrine HCl: 460 nm | 73 |
| 15 | Isoniazid and Lamivudine in marketed formulations | Spectrophotometric (Q-absorption ratio) | Phosphate buffer (pH7.4) | 5-30 | Iso-absorptive point: 246 nm Second wavelength: 272 nm | 74 |
| 16 | Isoniazid and Pyridoxine HCl in commercial tablets | Spectrophotometric (Area under curve) | Methanol | Isoniazid: 5-15 Pyridoxine HCl: 6–18 | Isoniazid: 262.2-272.2 nm Pyridoxine HCl: 289.8-299.8 nm | 75 |
| 17 | Isoniazid and Rifampicin from pharmaceutical preparations and biological fluids | Spectrophotometric Method A: Direct UV spectrophotometric measurement Method B: Reaction of drugs with N-bromosuccinimide (NBS) | Distilled water | Method A: Isoniazid: 2-42 Rifampicin: 0.822-65.38 Method B: Isoniazid: 0.1-3.4 Rifampicin: 0.5-15.9 | Method A: Isoniazid: 264 nm Rifampicin: 474 nm Method B: Isoniazid: 572 nm Rifampicin: 572 nm | 76 |
| 18 | Isoniazid Rifampicin and Piperine in pharmaceutical dosage form | Spectrophotometric (Absorption correction method) | Methanol and Distilled water | Isoniazid: 12-34.5 Rifampicin: 8-23 Piperine: 0.4-1.15 | Isoniazid: 262 nm Rifampicin: 477 nm Piperine: 338 nm | 77 |
| 19 | Isoniazid and Ethambutol HCl in pure form, pharmaceutical preparations and biological fluids | Spectrophotometric Method A (Reaction of Isoniazid with Iodine – starch solution Method B (Reaction of Isoniazid and Ethambutol hydrochloride with Hydroquinone solution) | Distilled water | Method A: Isoniazid: 1-6 Method B: Isoniazid: 2-100 Ethambutol HCl: 0.5-11.0 | Method A: Isoniazid: 572 nm Method B: Isoniazid: 310 nm Ethambutol HCl: 218 nm | 78 |
| | | **Rifampicin** | | | | |
| 1 | Rifampicin in bulk, capsule & spiked human urine | Spectrophotometric | Method A: 0.1 M HCl Method B: 0.1 M $H_3PO_4$ | 1.5-30 | Method A: 263 nm Method B: 259 nm | 79 |
| 2 | Rifampicin in bulk and capsule | Spectrophotometric | Methanol | 5-13 | 337 nm | 80 |
| 3 | Rifampicin in a | Spectrophotometric | Ethyl acetate | 2.5-35.0 | 344 nm | 81 |

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| 4 | mixture of Isoniazid and Pyrazinamide<br>Rifampicin pharmaceutical formulations | Visible Spectrophotometric | Buffer solution (pH=7.0) | 5-50 | 510 nm | 82 |
| | | | **Rifampicin in combination with other drugs** | | | |
| 5 | Rifampicin and Isoniazid in combined dosage form | Spectrophotometric (Simultaneous Equation Method) | Ethanol | Rifampicin: 5-35<br>Isoniazid: 5-25 | Rifampicin: 337 nm<br>Isoniazid: 263 nm | 83 |
| 6 | Rifampicin and Piperine in combined capsule dosage form | Spectrophotometric (Second order derivative) | Methanol | Rifampicin: 10-60<br>Piperine: 2-20 | ZCP for Rifampicin: 241 nm<br>ZCP for Piperine: 341 nm | 84 |
| 7 | Rifampicin and Piperine in combined capsule dosage form | Spectrophotometric (Dual Wavelength) | Methanol | Rifampicin: 10-60<br>Piperine: 1-10 | Rifampicin: 286 and 357 nm<br>Piperine: 356 nm and 479 nm | 85 |
| 8 | Rifampicin and Piperine in combined capsule dosage form | Spectrophotometric (Q-absorption ratio) | Methanol | Rifampicin: 5-40<br>Piperine: 2-20 | Iso-absorptive point: 387 nm<br>Second wavelength: 337 nm | 86 |
| 9 | Rifampicin & Isoniazid in urine and pharmaceutical formulation | Spectrophotometric (Multivariate Visible) | Deionized water | Rifampicin: 8-57<br>Isoniazid: 1.5-7 | Rifampicin: 449 nm<br>Isoniazid: 455 nm | 87 |
| | | | **Ethambutol** | | | |
| 1 | Ethambutol in pure form and in pharmaceutical formulations | Spectrophotometric (Using triphenyl methane dyes viz., Bromocresol Green (BCG), Bromocresol Purple (BCP) and Bromophenol Blue (BPB)) | Distilled Water | BCG: 2.0-25<br>BCP: 3.0-30<br>BPB: 4.0-40 | BCG: 420 nm<br>BCP: 419 nm<br>BPB: 415 nm | 88 |
| 2 | Ethambutol in pure form & pharmaceutical formulations | Spectrophotometric (based on reaction of the drug with 2,4-dinitro-1-fluorobenzene under stipulated conditions) | Distilled Water | 5-40 | 376 ± 1 nm | 89 |
| | | | **Pyrazinamide** | | | |
| 1 | Pyrazinamide in bulk and pharmaceutical dosage form | Spectrophotometric<br>Method A: Area under curve<br>Method B: Second order derivative | Water | Method A: 2-16<br>Method B: 2-16 | Method A: 264-274 nm<br>Method B: 270 nm | 90 |
| 2 | Pyrazinamide (PYN) and its impurity Pyrazine-2-carboxylic acid (PYA) | Spectrophotometric<br>Method A: Third order derivative<br>Method B: First order derivative | Methanol | PYN: 5-35<br>PYA: 5-30 | Method A:<br>PYN:276.2 nm<br>PYA:274.6 nm<br>Method B:<br>PYN:225.8 nm<br>PYA:245.2 nm | 91 |
| | | | **Pyrazinamide in combination with other drugs** | | | |
| 3 | Pyrazinamide (PYZ), Rifampicin (RIF) and Isoniazid (INH) in combined pharmaceutical dosage forms | Spectrophotometric (Second order derivative) | 0.1N HCl | PYZ: 5-15<br>RIF: 6-12<br>INH: 6-18 | PYZ:253.80 nm<br>RIF:299.80 nm<br>INH:302.40 nm | 92 |
| | | | **Rifabutin** | | | |
| 1 | Rifabutin in pharmaceutical formulations and in bulk drugs | Spectrophotometric<br>Method A: Quantitative precipitation of RFB with iodine<br>Method B: Quantitative precipitation of RFB with Tannic acid | Distilled water | Method A: 25-150<br>Method B: 10-60 | Method A: 520 nm<br>Method B: 460 nm | 93 |
| | | | **Rifapentine** | | | |
| 1 | Rifapentine in pure form and | Visible Spectrophotometric | 0.1N HCl | 5-50 | 478 nm | 94 |

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|---|---|---|---|---|---|---|
| | pharmaceutical formulations | | | | | |
| 2 | Rifapentine in bulk drug and tablets | Spectrophotometric (Area under curve) | Methanol | 4-24 | 334 nm | 95 |
| | | | **Ethionamide** | | | |
| 1 | Ethionamide in bulk, tablet and nanoparticles | Spectrophotometric | Phosphate buffer (pH 7.4) | 6-18 | 288 nm | 96 |
| 2 | Ethionamide in pharmaceuticals | Spectrophotometric | Methanol & water | 5-25 | 288 nm | 97 |
| 3 | Ethionamide in pharmaceuticals | Spectrophotometric | Water | 2.5-35 | 550 nm | 98 |
| 4 | Ethionamide in pharmaceuticals | Spectrophotometric Method A: Using Folin–Ciocalteu Method B: Using iron (III)-ferricyanide | 0.1 M HCl & Water | Method A: 1-40 Method B: 0.2-4 | Method A: 760 nm Method B: 760 nm | 99 |
| 5 | Ethionamide in pharmaceuticals | Spectrophotometric (Using two sulphonphthalein dyes) Method A: Using bromophenol blue Method B: Using bromothymol blue | Chloroform | Method A: 0.4-10 Method B: 0.5-14 | Method A: 450 nm Method B: 450 nm | 100 |
| | | | **Cycloserine** | | | |
| 1 | Cycloserine in bulk & capsule dosage form | Spectrophotometric Method A:Area under curve Method B: First order derivative | 0.01N HCl | 5-25 | Method A: 217 nm Method B: 217 nm | 101 |
| 2 | Cycloserine in pharmaceuticals | Spectrophotometric (Using Chloranil) | Borate buffer (pH 9) | 2-8 | 348 nm | 102 |
| | | | **Terizidone** | | | |
| 1 | Terizidone in bulk and capsule dosage form | Spectrophotometric Method A:Area under curve Method B: First order derivative | 0.1N NaOH | 4-12 | Method A: 273 nm Method B: 273 nm | 103 |
| | | | **p-Aminosalicylic Acid** | | | |
| 1 | p-Amino salicylic Acid in tablets | Spectrophotometric (Using derivatizing reagents) Method A: Using p-dimethylaminobenzaldehyde (DAB) Method B: Using p-dimethylaminocinnamaldehyde (DAC) | Ethanol Method A: 3M HCl-KCl buffer(pH 0.5) Method B: 5M HCl-KCl buffer (pH 0.5) | 0.4-2.0 | Method A: 460 nm Method B: 555 nm | 104 |
| | | | **Bedaquiline** | | | |
| 1 | Bedaquiline in bulk and pharmaceutical formulations | Spectrophotometric Method A: Zero order derivative Method B: Area under curve | Acetonitrile | 15-75 | 285 nm | 105 |

## 2. Spectrofluorimetric Methods:

**TABLE 6: SPECTROFLUORIMETRIC METHODS**

| S. no. | Drug / Sample | Method | Solvent | Linearity Range | Wavelength of Detection [λex/λem] | Ref. no. |
|---|---|---|---|---|---|---|
| | | | **Isoniazid** | | | |
| 1 | Isoniazid (INH), Ethambutol (EMB), Pyrazinamide (PZA) and Rifampicin (RIF) in pure and pharmaceutical dosage forms | Spectrofluorimetric (Based on measuring the quenching effect of studied drugs on the fluorescence intensity of NBS-phenothiazine oxidation product (NBS-Phz)) | Methanol | INH= 0.1-0.35 µg/ml EMB= 1-4 µg/ml PZA= 0.1-1 µg/ml RIF= 1-5 | 271/375 nm | 106 |
| | | | **Ethionamide** | | | |
| 1 | Ethionamide (ETN) & Carbocisteine (CBC) in their dosage forms | Spectrofluorimetric (Based on the reaction of drugs with roth's reagent (o-phthaldehyde) to get a | Distilled water | ETN= 0.25-2.5 CBC= 0.05-0.9 | ETN= 339/424 nm CBC= 329/431 nm | 107 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | highly fluorescent isoindole product) | | | | |
| | | | **p-Aminosalicylic Acid** | | | |
| 1 | p-Aminosalicylic acid | Spectrofluorimetric | sodium acetate buffer (pH 4.0) | 0.051-12 Mm | 297/394 nm | 108 |
| 2 | p-Aminosalicylic acids (PAS) & p-A minobenzoic (PABA) in biological fluids | Spectrofluorimetric (Using terbium-sensitized Luminescence) | Water | PAS: 0-40 µmol/L PABA:0- 10 µmol/L | In alkaline solution= 324/546 nm In acidic solution= 292/546 nm | 109 |

## 3. Chromatographic Methods:
## A. Liquid Chromatographic Methods:

**TABLE 7: LIQUID CHROMATOGRAPHIC METHODS**

| S. no. | Drug / Sample | Method | Column | Mobile phase | Flow Rate (ml/ min) | Detection | Ref. no. |
|---|---|---|---|---|---|---|---|
| | | | **Isoniazid** | | | | |
| 1 | Isoniazid in human plasma | HPLC | VP-ODS C18 (250 mm x 4.6 mm, 5 µm) | Aquabidest: Acetonitrile (97:3, v/v) | 1 | UV 262 nm | 110 |
| 2 | Isoniazid in plasma, brain, liver and kidney samples and in solid lipid nanoparticles | HPLC | Waters, Symmetry Shield RP-18 (150 mm x 4.6 mm, 5 µm) | 0.1 M phosphate buffer (pH 5 adjusted with ortho phosphoric acid) and methanol (50:50, v/v) | 0.9 | PDA 254 nm | 111 |
| 3 | Isoniazid in serum | HPLC | C18 (250 mm x 4.6 mm, 4 µm) | Acetonitrile,water, triethylamine & acetic acid (400:600:2:1, v/v/v/v) | 1 | UV 340 nm | 112 |
| 4 | Isoniazid in rat plasma | HPLC | C18 (150 mm x 4.6 mm, 5 µm) | Hexane sulphonate 20 mM (pH 2.47) & Methanol (65:35, v/v) | 1 | UV 265 nm | 113 |
| 5 | Isoniazid | HPLC | C18 (250 mm x 4.6 mm, 5 µm) | 5.3% ethanol, 93.7% water, 1% acetic acid | 1 | UV 265 nm | 114 |
| 6 | Isoniazid in human plasma | HPLC | Pinnacle II C18 (150 mm x 4.6 mm, 5 µm) | 0.05 M ammonium acetate buffer (pH 6): Acetonitrile (99:1, v/v) | 1.2 | UV 275 nm | 115 |
| | | | **Isoniazid in combination with other drugs** | | | | |
| 7 | Isoniazid & Rifampicin in bulk and pharmaceutical formulations | RP-HPLC | XDB C18 (150 mm x 4.6 mm, 5 µm) | $KH_2PO_4$ buffer (pH 4.5): Methanol (60:40, v/v) | 0.8 | PDA 258 nm | 116 |
| 8 | Isoniazid & Rifampicin in nanoparticle drug formulations | RP-HPLC | Phenomenex Luna C18 (150 mm x 4.6 mm, 5 µm) | Methanol and water (10:90, v/v) | 1 | UV 268 nm | 117 |
| 9 | Isoniazid & Omeprazole determination in human serum | HPLC | Octasilil C8 (250 mm x 4.6 mm, 5 µm) | 10 mM triethylamine pH 10.5: acetonitrile (67:33, v/v) | 1 | UV 260 nm | 118 |
| 10 | Isoniazid & Acetyl isoniazid in plasma | HPLC | ODS (150 mm × 3 mm, 3.5 µm) | 20 mM 1-hexanesulfonic acid sodium salt solution (pH 3 adjusted with phosphoric acid) and acetonitrile in gradient elution program | 0.4 | UV 290 nm | 119 |
| 11 | Isoniazid and Acetyl Isoniazid in urine | HPLC | C8 (250 mm x 4.6 mm, 5 µm) | Water and methanol (80:20, v/v) | 1.2 | PDA 274 nm | 120 |
| 12 | Isoniazid (INH) & Ciprofloxacin Hydrochloride encapsulated in lipid polymeric hybrid nanoparticles | RP-HPLC | C18 (150 mm x 4.6 mm, 5 µm) | 0.1% trifluoroacetic acid acetonitrile (70:30, v/v) | 1 | PDA 272 nm | 121 |
| 13 | Isoniazid and its related substances in | HPLC | C18 (250 mm x 4.6 mm, 5 µm) | A potassium dihydrogen orthophosphate buffer of pH | 1.5 | PDA 254 nm | 122 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Isoniazid and Ethambutol HCl tablet | | | 6.9 | | | |
| 14 | Isoniazid and Ethambutol in tablet dosage form | RP-HPLC | ODS C18 (250 mm x 4.6 mm, 5 µm) | 0.05M Phosphate buffer (pH 4.6) and Acetonitrile (30:70, v/v) | 1 | PDA 255 nm | 123 |
| 15 | Isoniazid -pyridoxine HCl mixture | HPLC | ODS (250 mm x 4.6 mm, 5 µm) | Methanol: water (60:40, v/v) | 2 | 293 nm | 124 |
| 16 | Isoniazid and Ethambutol in pharmaceuticals | HPLC | C18 Thermo Hypersil ODS, (250 mm x 5.4 mm, 4.5 µm) | Methanol: ammonium acetate buffer (pH-7.03) (50:50, v/v) | 1.3 | UV 276 nm | 115 |
| 17 | Isoniazid, Rifampicin in tablet dosage form | HPLC | Inertsil (250 mm x 4.6 mm, 5 µm) | Water (pH 4.5 adjusted with sodium dihydrogen phosphate): Acetonitrile (40:60, v/v) | 1 | UV 274 nm | 125 |
| 18 | Isoniazid, Ethambutol Hydrochloride & Rifampicin in tablet formulation | RP-HPLC | Prontosil C18 (250 mm x 4.6 mm, 5 µm) | Acetonitrile: 0.02M sodium dihydrogen phosphate buffer (pH 6.5 adjusted with orthophosphoric acid) (60:40, v/v) | 1 | UV 208 nm | 126 |
| 19 | Isoniazid, Thiacetazone and Pyridoxine HCl in tablet dosage form | RP-HPLC | Inertsil ODS Zodiac C18 (250 mm x 4.6 mm, 5 µm) | Ammonium Acetate: acetonitrile (30:70, v/v) | 1 | UV 254 nm | 127 |
| 20 | Isoniazid, Thiacetazone and Pyridoxine in tablet dosage form | RP-HPLC | Hypersil ODS C18 (150 mm x 4.6 mm, 5 µm) | Ammonium formate buffer: acetonitrile (60:40, v/v) | 1 | UV 254 nm | 128 |
| 21 | Isoniazid, Rifampicin & Piperine in pharmaceuticals | HPLC | E-Merck RP-18 (250 mmx 4.0 mm,5 µm) | Sol. A: Water + 0.1% acetic acid buffer, 2.5mM ammonium acetate Sol. B: Acetonitrile + 0.1% acetic acid buffer (10:90, v/v) | 0.4 | UV 263 nm | 115 |
| 22 | Isoniazid, Rifampicin, Piperine in pure & pharmaceutical dosage form | RP-HPLC | LC18 (250 mm x 4.6 mm, 5 µm) | 0.01M Sodium dihydrogen orthophosphate, pH 6.5 and acetonitrile (40:60, v/v) | 0.9 | PDA 282 nm | 77 |
| | | | | **Rifampicin** | | | |
| 1 | Rifampicin in complex pharmaceutical formulation and human serum | HPLC | Zorbax C18 (250 mm x 4.6 mm, 5 µm) | Methanol and water in gradient programme | 1 | UV 333.6 nm | 129 |
| 2 | Rifampicin in human plasma | HPLC | Phenomenex ODS C18 (150 mm x 4.6 mm, 5 µm) | Acetonitrile and 10mM potassium dihydrogen phosphate (pH adjusted to 3.2) (40:60, v/v) | 1 | UV 337 nm | 130 |
| 3 | Rifampicin in cerebrospinal fluid and plasma of the rabbit | HPLC | C8 (250 mm x 4.6 mm, 5 µm) | Acetonitrile: 10 mM phosphate buffer of pH 3.5 (48: 52, v/v) | 1 | 215 nm | 131 |
| 4 | Rifampicin in human plasma | HPLC | Chromolith RP8 column (100 mm x 4.6 mm, 2 µm) | 0.05 m acetate buffer pH 5.7: acetonitrile (35:65, v/v) | 1 | UV 335 nm | 132 |
| 5 | Rifampicin in dried bloods spots | HPLC | C-8 (Waters, Sunfire (250 mm x 4.6 mm, 5 µm) | 50 mM ammonium acetate buffer pH 4.5, Acetonitrile and Methanol (40:30:30, v/v/v) | 0.5 | UV 261 nm | 133 |
| 6 | Rifampicin in bulk and pharmaceutical dosage form | RP-HPLC | C18 (250 x 4.6 mm, 3.5 µm) | Acetonitrile and water (80:20, v/v) | 0.8 | UV 237 nm | 134 |
| 7 | Rifampicin in plasma | RP-HPLC | C18 (250 mm x 4.0 mm, 4 µm) | phosphate buffer pH 7.4: methanol (75:25, v/v) | 1.5 | UV 475 nm | 135 |
| 8 | Rifampicin in bulk form and capsules | UPLC | Waters Acquity UPLC BEH C18 (100 mm x 2.1 mm, 1.7 µm) | Milli-Q water and acetonitrile (50:50, v/v) | 0.4 | UV 235 nm | 136 |
| 9 | Rifampicin in human serum | UPLC | BEH C18 (100 mm x 2.1 mm, 1.7 µm) | Acetonitrile & 0.05 M acetate buffer pH 4.0 (35:65, v/v) | 0.5 | UV 334 nm | 137 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 10 | Rifampicin in human Plasma, broncho-alveolar lavage fluid and alveolar cells | HPLC | Ultrasphere octyl (150 mm x 4.6 mm, 5 µm) | 36% acetonitrile in water, 0.2% phosphoric acid, and 0.5% hydrogen peroxide adjusted to pH 4.5 with sodium hydroxide | 1 | Fluores-cence 380/490 nm | 138 |
| 11 | Rifampicin in serum | HPLC | Phenomenex Prodigy ODS (150 mm x 4.6 mm, 5 µm) | 0.1mmol/L phosphate buffer pH 4.8: methanol (70:30, v/v) | 1 | 335 nm | 82 |
| 12 | Rifampicin | RP-HPLC | ODS C18 (150 mm x 4.6 mm, 3.5 µm) | Potassium dihydrogen phosphate buffer (pH 3 adjusted with o-phosphoric acid) and acetonitrile (50:50, v/v) | 1 | PDA 238 nm | 82 |
| 13 | Rifampicin In tablet dosage form | RP-HPLC | C18 (250 mm x 4.6 mm, 5 µm) | Acetonitrile: 0.05M potassium phosphate buffer (38:62, v/v) | 1 | UV 335 nm | 125 |
| 14 | 25-Desacetyl Rifampicin (25-DR) in human urine | HPLC | Agilent Eclipse XDB C18 (250 mm x 4.6 mm, 5 µm) | Methanol: 0.01 M sodium phosphate buffer pH 5.2 (65:35, v/v) | 0.8 | 254 nm | 139 |
| | **Rifampicin in combination with other drugs** | | | | | | |
| 15 | Rifampicin and 25-desacetyl-rifampicin in plasma | HPLC | C18 (250 x 4.6 mm, 5 µm) | Methanol and 0.058 M sodium nitrite solution (63:37, v/v) | 4.7 | UV 335 nm | 140 |
| 16 | Rifampicin and 25-O-Desacetyl Rifampicin in vitro metabolism | HPLC | A Phenomenex Luna C-18 (150 mm × 4.6 mm, 5 µm) | Water & methanol in gradient elution program | 0.8 | PDA 254 nm | 141 |
| 17 | Rifampicin and desacetyl rifampicin in plasma and urine | HPLC | Phenomenex Luna C18 (250 mm x 4.6 mm, 5 µm) | 0.05 M phosphate buffer (pH 2.6): acetonitrile (55:45, v/v) | 1.2 | PDA 254 nm | 142 |
| 18 | Rifampicin and related compounds in pharmaceuticals | HPLC | C18 monolithic (100 mm x 4.6 mm, 5 µm) | Methanol, acetonitrile, 0.075 M monopotassium phosphate & 1.0 M citric acid (28:30:38:4, v/v) | 2 | UV 254 nm | 143 |
| 19 | Rifampicin & Clindamycin phosphate in skin permeation studies | HPLC | C18 (150 mm x 4.6 mm, 5 µm) | 0.01 M phosphoric acid and methanol in gradient elution program | 1 | UV 238 nm 200 nm | 144 |
| 20 | Rifampicin &Daptomycin in rabbit plasma | UPLC | Acquity BEH C18 (100 mm x 2.1 mm, 1.7 µm) | Methanol and 0.1% aqueous TFA in gradient elution program | 1 | UV | 145 |
| 21 | Rifampicin and a flavonoid glycoside | RP-HPLC | RP-18 (250 mm × 4.6 mm, 5 µm) | Acetonitrile & 50 mM phosphate buffer (pH 5.0) (60:40, v/v) | 0.8 | DAD 340 nm | 146 |
| 22 | Rifampicin and Sulbactam in mouse plasma | HPLC | RP-18 (125 mm × 4.0 mm, 5 µm) | 50 mM potassium dihydrogen phosphate solution (pH 4.5) and acetonitrile in gradient elution program | 1 | DAD 230 nm | 147 |
| 23 | Rifampicin and Piperine in pharmaceutical dosage form | RP-HPLC | C18 (250 mm x 4.6 mm, 5 µm) | Potassium dihydrogen orthophosphate pH 6.5 and acetonitrile (30:70, v/v) | 1 | PDA 341 nm | 148 |
| 24 | Rifampicin and Ofloxacin in synthetic mixture | HPLC | Kinetex C18, Phenomenex (250 mm x 4.6 mm, 5 µm) | 0.03M Potassium dihydrogen phosphate buffer pH 3.0: acetonitrile (55:45, v/v) | 0.8 | PDA 230 nm | 149 |
| 25 | Rifampicin and Isoniazid in human plasma | HPLC | 1.Luna C18 (250 mm x 4.6 mm, 5 µm) 2.Luna C8 (250 mm x 4.6 mm, 5 µm) | 1.Methanol:0.02M Potassium phosphate buffer pH 7.0 (75:25, v/v) 2.Methanol: water: perchloric acid: tetrabutylammonium hydroxide solution (20:80:0.05:0.05, v/v/v/v) | 0.5 & 1 | PDA 339 nm 273 nm | 150 |
| 26 | Rifampicin and Isoniazid in pharmaceutical formulations | RP-HPLC | Kromasil C18 (250 mm x 4.6 nm, 5 µm) | Methanol, acetonitrile and water (60:20:20, v/v/v) | 1 | UV 254 nm | 151 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 27 | Rifampicin & Hydro-chlorothiazide | HPLC | Phenomenex ODS 2 C18 (150 mm x 4.6 mm, 5 µm) | Acetonitrile and 10mM $KH_2PO_4$ (pH 3.2) (40:60, v/v) | 1 | 337 nm | 82 |
| 28 | Rifampicin and Isoniazid | HPLC | ODS (250 mm x 4.6 nm, 5 µm) | Methanol:0.02M disodium hydrogen orthophosphate (75:25, v/v) | 1 | 254 nm | 82 |
| 29 | Rifampicin & isoniazid | HPLC | C18 (250 mm x 4.6 mm, 5 µm) | 0.05M sodium dihydrogen phosphate (pH 3.1) and acetonitrile (20:80, v/v) | 0.6 | 254 nm | 82 |
| | | | **Ethambutol** | | | | |
| 1 | Ethambutol in human plasma | HPLC | CN (150 mm x 4.6 mm, 5 µm) | Milli-Q water and methanol (85:15, v/v) | 1.5 | PDA 267 nm | 152 |
| 2 | Ethambutol in serum | LC | Waters C18 (150mm x 4.6 mm, 5 µm) | Aqueous 72% (v/v) acetonitrile | 1 | Fluore-scence 345/475 nm | 153 |
| 3 | Ethambutol in rat plasma | UPLC | BEH RP 18 (50 mm x 2.1 mm, 1.7 µm) | Methanol and water (70: 30, v/v) | 0.1 | PDA 205 nm | 154 |
| 4 | Ethambutol in pharmaceutical dosage form | RP-HPLC | C18, (50 mm x 4.6 mm, 5 µm) | Dichloromethane: methanol: formic acid (70:30:0.1, v/v/v) | 1 | 225 nm | 125 |
| | | | **Ethambutol in combination with other drugs** | | | | |
| **5** | Ethambutol Hydrochloride and Isoniazid in fixed dose formulation | RP-HPLC | C18 Thermo Hypersil ODS (250 mm x 5.4mm, 4.5 µm) | Methanol: ammonium acetate buffer (pH-7.03)(50:50, v/v) | 1.3 | PDA 276 nm | 155 |
| | | | **Pyrazinamide** | | | | |
| 1 | Pyrazinamide in human plasma | HPLC | Supelco LC-18 (150 mm x 4.6 mm, 5 µm) | 0.02 M phosphate buffer (pH 7.4) & methanol (96.8:3.2, v/v) | 1.5 | UV 268 nm | 156 |
| 2 | Pyrazinamide in human plasma | HPLC | Phenomenex ODS C18 (150 mm x 4.6 mm, 5 µm) | Methanol: potassium dihydrogen phosphate buffer (pH 7.4) (15:85, v/v) | 1 | UV 268 nm | 157 |
| 3 | Pyrazinamide in human plasma | HPLC | ODS C18 (250 mm x 4.6 mm, 5 µm) | Aquabidest: Acetonitrile (97:3, v/v) | 1 | UV 262 nm | 158 |
| 4 | Pyrazinamide in human plasma, bronchoalveolar lavage, and alveolar cells | HPLC | ODS C18 (250 mm x 4.6 mm, 5 µm) | 2.0% acetonitrile in 0.02M KH2PO4, adjusted to pH 2.6 with phosphoric acid | 1 | UV 268 nm | 159 |
| 5 | Pyrazinamide in bulk and pharmaceutical dosage forms | HPLC | Hypersil C8 (250 mm x 4.6 mm, 3.5 µm) | Phosphate buffer (pH 4.4): methanol (80:20, v/v) | 1 | UV 269 nm | 160 |
| 6 | Pyrazinamide in tablet dosage form | RP-HPLC | C18 (250 mm x 4.6 mm, 5 µm) | Acetonitrile and 15mM potassium dihydrogen (pH 4.0 ± 0.1 adjusted with o-phosphoric acid) (11:89, v/v) | 1 | 235 nm | 125 |
| 7 | Pyrazinamide in bulk and formulation | UHPLC | C18 (25 mm x 4.6 mm, 1.7 µm) | Phosphate buffer: acetonitrile (900:100, v/v) | 1 | PDA 270 nm | 161 |
| 8 | Pyrazinamide in pharmaceutical formulation | Micellar LC | SPHER-100 C18 (250 mm x 4.6 mm, 5 µm) | 0.15M sodium dodecyl sulphate and 1% butanol (v/v) buffered at pH3 in gradient elution program | 1 | UV 269 nm | 162 |
| | | | **Pyrazinamide in combination with other drugs** | | | | |
| 9 | Pyrazinamide and Rifampicin in serum | HPLC | C8 (250 mm x 4.6 mm, 3.5 µm) | Acetonitrile in 10 mM potassium dihydrogen phosphate (pH 3.5) in gradient elution program | 1.5 | 215 nm | 163 |
| 10 | Pyrazinamide and Isoniazid in plasma | HPLC | Zorbax Eclipse Plus C18 (150 mm x 4.6 mm, 5 µm) | Acetonitrile and 20 mM 1-hexane sulfonic acid sodium salt (pH 2.7 adjusted with 10 % ortho-phosphoric acid) in gradient elution program | 1 | UV 269 nm & 340 nm | 164 |
| 11 | Pyrazinamide and Isoniazid in plasma | HPLC | Wakosil C18 HG (250 mm x 4.6 mm, 5 µm) | Acetonitrile and 0.05M ammonium acetate solution (1:99, v/v) | 1.2 | UV 275 nm | 165 |

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|---|---|---|---|---|---|---|---|
| 12 | Pyrazinamide and Isoniazid in plasma | HPLC | C8 (250 mm x 4.6 mm, 5 µm) | Water: methanol (80:20, v/v) | 1.5 | UV 267 nm | 166 |
| 13 | Pyrazinamide and Isoniazid in synthetic mixture | RP-HPLC | Inertsil-ODS C18 (250 mm x 4.6 mm, 5 µm) | Methanol: buffer (pH 4 adjusted with triethylamine) (55:45, v/v) | 1 | UV 267 nm | 167 |
| 14 | Pyrazinamide & Ethionamide from their porous microparticles | HPLC Ion-Pair | Phenomenex Luna C18 (250 mm x 4.6 mm, 5 µm) | 0.01% TFA in water and ACN/MeOH (50:50, v/v) in gradient elution program | 1.5 | UV 280 nm | 168 |
| 15 | Pyrazinamide, Rifampicin and Isoniazid in combined dosage forms | HPLC | YMC-ODS (150 mm x 4.6 mm, 5 µm) | Water, monobasic potassium dihydrogen orthophosphate and acetonitrile (900:60:40, v/v/v) | 1.5 | UV 254 nm | 169 |
| 16 | Pyrazinamide, Rifampicin and Isoniazid in pharmaceutical preparations | HPLC | Phenomenex C18 (250 mm x 4.6 mm, 5 µm) | Methanol, water, isopropanol, acetonitrile & 1mM sodium acetate (51:42:3:2:2, v/v/v/v/v) | 1.7 | UV 333 nm | 170 |
| 17 | Pyrazinamide, Rifampicin and Isoniazid in 0.1M HCl dissolution Medium and Simulated Gastric Fluid | HPLC | Suspelcosil LC18 (250 mm x 4.6 mm, 5 µm) | Methanol and 0.01M sodium dihydrogen orthophosphate buffer containing 0.05% tetramethyl-ammonium chloride (pH 3.5 adjusted with dil. orthophosphoric acid) in gradient elution program | 1 | UV 254 nm | 171 |
| 18 | Pyrazinamide, Rifampicin and Isoniazid in fixed dose combination | HPLC | µ-bondapak C18 (250-mm x 4.6 mm, 10 µm) | ACN:0.0002M tBAH (42.5:57.5, v/v) | 1 | UV 260 nm | 172 |
| 19 | Pyrazinamide (PYZ) Rifampicin (RIF) & Isoniazid (INH) in plasma | HPLC | For PYZ &INH Spherisorb C8 (150 mm x 4.6 mm, 5 µm) For RIF Spherisorb C8 (250 mm x 4.6 mm, 5 µm) | For PYZ &INH: 3% acetonitrile in 0.06% TFA For RIF: 80% acetonitrile in 0.1% trifluoroacetic acid | 2 & 1.5 | For PYZ &INH UV 254 nm For RIF UV 270 nm | 173 |
| 20 | Pyrazinamide, Rifampicin and Isoniazid in human plasma | RP-HPLC | Phenomenex ODS C18 (250 mm x 4.0 mm, 5 µm) | Acetonitrile, methanol andwater (pH5.2) (30:5:65, v/v/v) | 1 | UV 242 nm | 174 |
| 21 | Pyrazinamide, Rifampicin and Isoniazid in solid lipid nanoparticles | RP-HPLC | ODS C18 (250 mm x 4.0 mm, 5 µm) | A.OPA buffer (pH 6.8 ± 0.02 with dil. NaOH): acetonitrile (96:4, v/v) B. OPA buffer (pH 6.8 ± 0.02 with dil.NaOH): acetonitrile (45:55, v/v) Mobile phase A & B in gradient elution program | 1.5 | PDA 238 nm | 175 |
| 22 | Pyrazinamide, Isoniazid and Indomethacin in pharmaceutical preparation | HPLC | YMC-ODS (150 mm x 4.6 mm, 5 µm) | Water, methanol & tetrahydrofuran (59:39:2, v/v/v) | 2 | UV 328 nm | 176 |
| 23 | Pyrazinamide, Rifampicin and Isoniazid in tablet dosage form | RP-HPLC | Hypersil C18 (250 mm x 4.6 mm, 5 µm) | 0.05 M potassium phosphate buffer (pH 6.0): Methanol (40:60, v/v) | 1 | UV 254 nm | 125 |
| 24 | Pyrazinamide (PZA), Rifampicin (RIF) Isoniazid (INH) & Acetyl-isoniazid (AcINH) in Human Plasma | HPLC | Synergi Max-RP C12 (250 mm x 4.6 mm, 4 µm) | Methanol, acetonitrile and buffer of 20 mM 1-heptanesulfonic acid sodium (pH2.5 adjusted with $H_3PO_4$) in gradient elution program | 0.8, 1.2, 1.5 | DAD PZA= 268 nm AcINH= 265 nm INH= 264 nm RIF= 341 nm | 177 |
| 25 | Pyrazinamide, Rifampicin, Isoniazid | HPLC | Waters Symmetry C8 (250 mm x 4.6 | Acetonitrile and 20 mM phosphate buffer (pH | 1.5 | UV 210 nm | 178 |

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|---|---|---|---|---|---|---|---|
| | & Ethambutol HCl in fixed dose combination tablet | | mm, 5 µm) | 6.8)containing triethylamine in gradient elution program | | | |
| 26 | Pyrazinamide, Rifampicin, Isoniazid & Ethambutol HCl in fixed dose combination tablet | RP-HPLC | Waters Xterra RP18 (250 mm x 4.6 mm, 5 µm) | phosphate buffer (pH 6.8), 8% acetonitrile and acetate buffer (pH 4.7) in gradient elution program | 1 | UV 260 nm | 125 |
| 27 | Pyrazinamide (PYZ), Rifampicin (RIF), Isoniazid (INH), and Ethambutol hydrochloride (EMB) in fixed dose combination tablet | HPLC | Purospher STAR RP18e (250 mm x 4.6 mm, 5 µm) | 20 mM monobasic sodium phosphate buffer with 0.2% triethylamine (pH 7.0) and acetonitrile in gradient elution program | 1.5 | DAD PYR, RIF & INH = 238 nm EMB = 210 nm | 179 |
| 28 | Pyrazinamide (PYZ), Rifampicin (RIF), Isoniazid (INH) and Ethambutol hydrochloride (EMB) in fixed dose combination tablet | HPLC | Acclaim Polar Advantage II (150 mm x 4.6 mm, 3 µm) | A: 8% Acetonitrile in 20 mM $NaH_2PO_4$ (plus 1.5 mL TEA per liter), pH 6.8 B: 50% Acetonitrile in 20 mM $NaH_2PO_4$ (plus 1.5 mL TEA per liter), pH 6.8 | 1 | UV Channel-1 200 nm & 337 nm Channel-2 238nm | 180 |
| 29 | Pyrazinamide (PYZ), Rifampicin (RIF), Isoniazid (INH), and Ethambutol hydrochloride (EMB) in fixed dose combination tablet | UHPLC | Acclaim Polar Advantage II (100 mm x 2.1 mm, 2.2 µm) | A: 4% Acetonitrile in 20mM $NaH_2PO_4$ (plus 1.5 mL TEA per liter), pH 6.8 B: 50% Acetonitrile in 20 mM $NaH_2PO_4$ (plus 1.5 mL TEA per liter), pH 6.8 | 1 | UV Channel-1 200 nm & 337 nm Channel-2 238nm | 180 |
| 30 | Pyrazinamide (PYZ), Rifampicin (RIF), Isoniazid (INH), and Ethambutol hydrochloride (EMB) in fixed dose combination tablet | UHPLC | Waters Acquity BEH C18 (50 mm x 2.1 mm, 1.7 µm) | Triethylamine in phosphate buffer pH 6.8 and acetonitrile (95:5, v/v) | 0.4 | UV PYR, RIF & INH = 238 nm EMB = 210 nm | 181 |
| 31 | Pyrazinamide, Rifampicin, Isoniazid & Ethambutol HCl in fixed dose combination tablet | UPLC | X bridge C18 (50 mm x 1.7 mm, 3 µm) | Solution-A: Triethylamine and potassium dihydrogen ortho-Phosphate buffer (pH 7.5 adjusted with ortho phosphoric acid) Solution-B: Mixture of methanol and acetonitrile (85:15, v/v) -Mixture of solution- A and solution-B (90:10, v/v) | 0.5 | PDA 290 nm | 182 |
| 32 | Pyrazinamide, Rifampicin, Isoniazid & Pyridoxine HCl in pharmaceutical formulation | RP-HPLC | Phenomenex Luna C18 (250 mm x 4.6 mm, 5 µm) | Acetonitrile and 15 mmol/L potassium dihydrogen phosphate buffer (pH 4.0 ± 0.1 adjusted by orthophosphoric acid) in gradient elution program | 1 | PDA 235 nm | 183 |
| | | | | **Rifabutin** | | | |
| 1 | Rifabutin in human plasma | HPLC | C18 (250 mm x 4.6 mm, 5 µm | 50mM phosphate buffer, (pH 4.2 adjusted with 1N HCl) & acetonitrile (53:47, v/v) | 1.2 | UV 265 nm | 184 |
| 2 | Rifabutin | HPLC | C18 (250 mm x 4.6 mm, 5 µm | Acetonitrile + methanol (1:1): water (75:25, v/v) | 1 | UV 242 nm | 185 |
| 3 | Rifabutin in bulk dosage form | RP-HPLC | Phenomenex C8 Luna (250 mm x 4.6 mm, 5 µm) | Methanol and water (75:25 v/v) | 1 | UV 240 nm | 186 |

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|---|---|---|---|---|---|---|---|
| 4 | Rifabutin in bulk drugs and pharmaceutical dosage form | Stability LC | Ace5-C18 (250 mm x 4.6 mm, 5 µm) | 50 mM ammonium acetate (pH 4 adjusted by acetic acid) and acetonitrile (50:50, v/v) | 1 | UV 275 nm | 187 |
| 5 | Rifabutin in human plasma | HPLC | Zorbax C8 (250 mm x 4.6 mm, 5 µm) | 0.05 M potassium dihydrogen phosphate0.05 M sodium acetate at pH 4.0: acetonitrile (53:47, v/v) | 1 | UV 275 nm | 188 |
| | | | **Rifabutin in combination with other drugs** | | | | |
| 6 | Rifabutin and 25-O-desacetyl rifabutin in human plasma and urine | HPLC | ODS (250 mm x 4.6 mm, 5 µm) | Acetonitrile, 0.05 M potassium phosphate (pH 4.2) & Triethylamine (38:61.5:0.5, v/v/v) | 1 | UV 275 nm | 189 |
| | | | **Rifapentine** | | | | |
| 1 | Rifapentine in bulk and pharmaceutical dosage form | RP-HPLC | Inertsil C18 (250 mm x 4.6 mm, 5 µm) | Acetonitrile and 0.01M potassium dihydrogen phosphate buffer, pH (6.0), (80:20, v/v) | 0.8 | UV/Visible 478 nm | 190 |
| 2 | Rifapentine | HPLC Impurity profile | BDS-Hypersil C18 (250 mm x 4.6 mm, 5 µm) | A mixture of 0.025M sodium dihydrogen orthophosphate buffer (pH 7.7 adjusted with dil. NaOH) and ACN (90:10, v/v) for mobile phase A (30:70, v/v) for mobile phase B. Use Mobile phase A & B in gradient elution program | 1 | PDA 254 nm | 191 |
| | | | **Ethionamide** | | | | |
| 1 | Ethionamide in human plasma | HPLC | CN (150 mm x 4.6 mm, 5 µm) | Milli-Q water and methanol (85:15, v/v) | 1.5 | PDA 267 nm | 192 |
| 2 | Ethionamide in Serum | HPLC | Hypersil ODS C18 (250 mm x 4.6 mm, 5 µm) | 0.02 M disodium hydrogen phosphate buffer: acetonitrile (75:25, v/v) | 1.5 | UV 291 nm | 193 |
| 3 | Ethionamide in dosage form | RP-HPLC | Hypersil BDS C18 (150 mm x 6 mm, 3 µm) | Acetonitrile: water (30:70, v/v) | 1 | UV 287 nm | 194 |
| 4 | Ethionamide in raw material & pharmaceutical dosage forms | Stability HPLC | ODS C18 (250 mm x 4.6 mm, 5 µm) | Acetonitrile: 0.05% trifluoroacetic acid solution (30:70, v/v) | 0.8 | UV 270 nm | 195 |
| 5 | Ethionamide in spiked human plasma | RP-HPLC | C18 (250 mm x 4.6 mm, 5 µm) | Methanol: water (40:60, v/v) | 1 | UV 275 nm | 196 |
| 6 | Ethionamide in pharmaceutical dosage forms | RP-HPLC | Grace C18 (250 mm x 4.6 mm, 5 µm) | Methanol: 0.1% Ortho Phosphoric acid (20:80, v/v) | 0.7 | UV 288 nm | 97 |
| | | | **Ethionamide in combination with other drugs** | | | | |
| 7 | Ethionamide, Pyridoxine, and Moxifloxacin in fixed dose combination tablets | Stability RP-HPLC | Hibar RP 18 (150 mm x 4.6 mm, 5 µm) | 0.03M sodium citrate buffer (pH 5.0 adjusted with glacial acetic acid) and methanol in gradient elution program | 1 | UV 320 nm | 197 |
| | | | **Cycloserine** | | | | |
| 1 | Cycloserine in human plasma | HPLC | C18 (250 mm x 4.6 mm, 5 µm) | 0.1% formic acid solution and a mixture of methanol and acetonitrile (1:1) (85:15, v/v) | 1 | Fluore-scence 381/450 nm | 198 |
| 2 | D-Cycloserine & related substance | LC | Hypersil BDS C18 (250 mm x 4.6 mm, 5 µm) | Acetonitrile, 20mM sodium octane sulphonate, 0.2M potassium dihydrogen phosphate buffer pH 2.8 & water in gradient elution program | 1 | UV 219 nm | 199 |
| 3 | D-Cycloserine drug substance | RP-HPLC | Agilent Zorbax SB phenyl (250 mm x 4.6 mm, 5 µm | 20mM $Na_2HPO_4$ (pH 7 adjusted with ortho-phosphoric acid) and acetonitrile (95:5, v/v) | 1 | UV 335 nm | 200 |
| | | | **Terizidone** | | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 1 | Terizidone | Stability RP-HPLC | HiQSil C8 (250 mm x 4.6 mm, 5 µm) | Ammonium acetate buffer (pH 3 adjusted with glacial acetic acid) and methanol (60:40, v/v) | 1 | PDA 264 nm | 201 |
| 2 | Terizidone in plasma | HPLC | HS C18 (150 mm x 4.6 mm, 5 µm) | Acetonitrile and water both containing 0.1% formic acid in gradient elution program | 1 | UV 264 nm | 202 |
| | | | | **p-Aminosalicylic Acid** | | | |
| 1 | p-Aminoslicylic acid and its metabolite in plasma, cerebrospinal fluid and brain tissues | HPLC | C18 (250 mm x 4.6 mm, 5 µm) | 17.5 mM potassium phosphate buffer (equal molar concentration of both monobasic and dibasic potassium salts with a pH of 3.5 adjusted by phosphoric acid) and methanol in gradient elution program | 1 | Fluorescence 337/432 nm | 203 |
| 2 | p-Aminosalicylic acid (PAS) and its degradation product m-aminophenol (MAP) in pellets | Ion-pair HPLC | LiChrospherRP 18 (125 mm x 4 mm, 5µm) | 20 mM phosphate buffer, 20 mM tetrabutylammonium hydrogen sulphate & methanol (16%, v/v) (pH 6.8) gradient elution program | 1 | UV 233 nm | 204 |
| | | | | **Bedaquiline** | | | |
| 1 | Bedaquiline | RP-HPLC | Chiralcel OJ-3R (cellulose tris-[4-methylphenyl]benzoate, 150 mm x 4.6 mm, 3 µm) | 10 mM buffer of triethylamine/phosphoric acid pH 7.0 and acetonitrile (40:60, v/v) | 0.1-1.4 | UV 227 nm | 205 |
| | | | | **Bedaquiline in combination with other drugs** | | | |
| 2 | Bedaquiline (BED), Moxifloxacin (MOX) & Pyrazinamide (PYZ) in pharmaceutical powder formulation for inhalation | RP-HPLC | Luna C18 (150 mm x 4.6 mm, 5 µm) | Methanol and triethylamine phosphate buffer (pH 2.5) in gradient elution program | 1.2 | PDA BED= 225 nm MOX= 296 nm PYZ= 269 nm | 206 |
| | | | | **Pretomanid** | | | |
| 1 | Pretomanid (PA-824), Moxifloxacin (MOX) and Pyrazinamide (PYZ) in an inhaler | HPLC | Luna C18 (150 mm x 4.6 mm, 5 µm) | Methanol and trimethylamine phosphate buffer (pH 2.5) in gradient elution program | 1 | PDA PRM= 330 nm MOX= 296 nm PYZ= 269 nm | 207 |

## B. Thin Layer Chromatographic Methods:

**TABLE 8: THIN LAYER CHROMATOGRAPHIC METHODS**

| S. no. | Drug / Sample | Method | Stationary Phase | Mobile phase | Retention factor ($R_f$) | Detection | Ref. no. |
|---|---|---|---|---|---|---|---|
| | | | | **Isoniazid in combination with other drugs** | | | |
| 1 | Isoniazid (INH) and Acetyl isoniazid (AcINH) in serum | HPTLC | Silica gel 60 | Ethyl Acetate: methanol (70:30, v/v) | INH= 0.35 AcINH= 0.5 | 254 nm | 208 |
| 2 | Isoniazid (INH) and Rifampicin (RIF) in bulk drugs and formulations | Stability indicating HPTLC | Silica gel 60 F254 | n-hexane, 2propanol, acetone, ammonia, formic acid, (3:3.8:2.8:0.3:0.1, v/v/v/v/v) | INH = 0.59±0.02 RIF= 0.73±0.04, | 254 nm | 209 |
| 3 | Isoniazid (INH) and Rifabutin (RFB) in pharmaceutical formulation | Stability indicating HPTLC | Silica gel 60 F254 | Dichloromethane, acetone, methanol (20:7:2, v/v/v) | INH= 0.48±0.01 RFB= 0.84±0.01 | INH= 262 nm RFB= 504 nm | 210 |
| 4 | Isoniazid (INH), Pyridoxine hydrochloride (PYR) and Rifampicin (RIF) in combined | HPTLC | Precoated silica gel 60 G F254 aluminium | Ethyl acetate: methanol: acetone: acetic acid (5.5: 2.0: 2.0: 0.5, v/v/v/v) | INH= 0.47±0.01 PYR= 0.75±0.01 RIF= 0.27±0.01 | 254 nm | 211 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | tablet dosage form | | sheet | | | | |
| **Rifampicin in combination with other drugs** | | | | | | | |
| 1 | Rifampicin (RIF) and Isoniazid (INH) in rat plasma | HPTLC | Silica gel 60 F254 | Chloroform: methanol (9:1, v/v) | RIF= 0.27±0.01 INH= 0.47±0.01 | RIF= 475 nm INH= 280 nm | 212 |
| **Pyrazinamide in combination with other drugs** | | | | | | | |
| 1 | Pyrazinamide (PYN) and its impurity Pyrazine-2-carboxylic acid (PYA) | HPTLC | Silica gel 60 F254 | Methylenechloride: methanol: ammonia solution (7:3:0.1, v/v/v) | PYN= 0.86 PYA= 0.16 | 275 nm | 91 |
| 2 | Pyrazinamide (PYZ), Rifampicin (RIF) & Isoniazid (INH)in a fixed dosagecombination tablet | HPTLC | Silica gel 60 F254 plates | Acetate, acetone, methanol, glacial acetic acid with the ratio of (18:5:5:2, v/v/v/v) | PYZ=0.74 RIF=0.25, INH=0.44 | 277 nm | 213 |
| **Terizidone** | | | | | | | |
| 1 | Terizidone in pharmaceutical dosage form | HPTLC | Silica gel 60 F254 | Toluene: n-butanol (9:1, v/v) | 0.60±0.03 | 268 nm | 214 |

## 4. Gas Chromatography:

**TABLE 9: GAS CHROMATOGRAPHY**

| S. no. | Drug / Sample | Description | Ref. no. |
|---|---|---|---|
| **Isoniazid in combination with other drugs** | | | |
| 1 | Isoniazid (INH) and Hydrazine (HZ) in pharmaceutical preparations & blood | Capillary column gas chromatography after precolumn derivatization with trifluoroacetylacetone (FAA). Phenylhydrazine (PHZ) when present together with INH and HZ also separated completely from the column HP-5 (30 mm x 0.32 mm) connected with flame ionization detection (FID). The solvent was evaporated under nitrogen gas and re-dissolved in 0.2 mL of methanol. The total run time was 7 min and nitrogen flow rate was 1mL/min. The linear calibration ranges for INH and HZ were determined to be 2.5-25 µg/mL and 2.5-21.2 µg/mL respectively, the detection limits were obtained at 62.5 pg reaching to the detector. | 215 |

## 5. Micellar Electrokinetic Capillary Chromatography:

**Table 10: MICELLAR ELECTROKINETIC CAPILLARY CHROMATOGRAPHY**

| S. no. | Drug / Sample | Method | Stationary Phase | Mobile phase | Flow Rate (ml/ min) | Detection | Ref. no. |
|---|---|---|---|---|---|---|---|
| **Isoniazid in combination with other drugs** | | | | | | | |
| 1 | Isoniazid (INH), Pyrazinamide (PYR) and Rifampicin (RIF) in pharmaceutical products | MEKC | Nova-Pak C18 (150 mm x 3.9 mm, 4 µm) | Methanol in 20mM phosphate buffer & methanol in gradient elution program | 1 | 254 nm | 216 |

## 6. Electrochemical Methods:

**Table 11: ELECTROCHEMICAL METHODS**

| S. no. | Drug/ Sample | Method | Electrode | | Linearity Range | LOD | Ref. no. |
|---|---|---|---|---|---|---|---|
| | | | Working Electrode | Reference Electrode | | | |
| **Isoniazid** | | | | | | | |
| 1 | Isoniazid | Voltammetry (Differential Pulse Voltammetry) | Mercury film silver-based electrode (Hg (Ag) FE) | Ag/AgCl/ KCl | 5-500 nM | 4.1 nM | 217 |
| 2 | Isoniazid in urine | Amperometry | Glassy carbon electrode | Ag/AgCl/ KCl | 0.05-783.1 µM | 0.01 µM | 218 |
| 3 | Isoniazid | Voltammetry (Using poly (3,4-ethylenedioxythiophene)-modified gold electrode) | Crystalline Au (111) | Ag/AgCl/3M NaCl | 0.05-2 µM | 0.014 µM | 219 |
| 4 | Isoniazid in tablets | Amperometric | Glassy carbon electrode | Ag/AgCl | $2.5 \times 10^{-8}$ - $1.0 \times 10^{-3}$ M | $4.1 \times 10^{-9}$ M | 220 |
| **Isoniazid in combination with other drugs** | | | | | | | |
| 5 | Isoniazid (INH) and | Voltammetry | Hanging mercury drop | Ag/AgCl/ | INH= 0.25- | INH= | 221 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Rifampicin (RIF) in pharmaceutical formulations | (Differential Pulse Voltammetry) | electrode (HMDE) | KCl | 1.25 mg/L RIF= 0.40-2.00 mg/L | 0.05 mg/L RIF= 0.07 mg/L | |
| 6 | Isoniazid (INH) & Acetaminophen (AAP) in human fluids | Voltammetry | Bismuth oxide modified screen-printed electrode | Ag/AgCl | INH= 5-1760 µM AAP= 0.5-1250 µM | INH= 1.85 µM AAP= 30 nM | 222 |
| | | | **Pyrazinamide** | | | | |
| 1 | Pyrazinamide | Voltammetry | Screen-printed carbon electrode (SPCE) | Ag/AgCl | $9.0 \times 10^{-7}$ - $1.0 \times 10^{-4}$ mol /L | $5.7 \times 10^{-7}$ mol/L | 223 |
| | | | **Ethionamide** | | | | |
| 1 | Ethionamide in Pharmaceutical Formulations | Voltammetry | Boron-doped diamond electrode | Ag/AgCl | 1.00-80.0 µmol/ L | 0.294 µmol/L | 224 |
| | | | **Ethionamide in combination with other drugs** | | | | |
| 2 | Ethionamide (ETH) and Pyrazinamide (PYZ) | Voltammetry | Glassy carbon electrode | Ag/AgCl | ETH= 2.38-248.0 µmol /L PYZ= 0.476-51.2 µmol/L | ETH= 0.531 µmol /L PYZ= 0.113 µmol /L | 225 |
| | | | **Cycloserine** | | | | |
| 1 | D-Cycloserine in pharmaceutical and human biological samples | Voltammetry | Gold electrode | Ag/AgCl/ KCl | 0.1-1.1 µM | $3.3 \times 10^{-8}$ M | 226 |
| 2 | D-Cycloserine in pharmaceutical products | Voltammetry (Stair Case (SCV) and Square Wave (SWV) | Graphene paste electrode | Ag/AgCl | SCV= $1.0\times10^{-8}$ - $1.5\times10^{-7}$ M SWV= $1.0\times10^{-8}$ - $1.1\times10^{-7}$ M | SCV= 2.80 nM SWV= 3.70 nM | 227 |

## 7. Titrimetric Methods:

**TABLE 11: TITRIMETRIC METHODS**

| S. no. | Drug / Sample | Description | Ref. no. |
|---|---|---|---|
| | | **Isoniazid** | |
| 1 | Isoniazid | N-bromophthalimide used as a titrant. The end-point is determined either directly using methyl red or amaranth as indicator, or by a back-titration method in which a known excess of N-bromophthalimide solution is added to isoniazid solution and then the residual unreacted reagent is determined iodometrically. | 70 |
| 2 | Isoniazid | Titration of isoniazid with 0.02 M acetous perchloric acid in glacial acetic acid using crystal violet as an indicator. The method is applicable over the range of 1.5-15 mg isoniazid | 228 |
| | | **Ethambutol** | |
| 1 | Ethambutol | 0.2 gm of pure powder or 0.2 gm equivalent of ethambutol hydrochloride powder (in case of tablet) was taken in a 250 ml separating flask and 10 ml of 2N sodium hydroxide was added to the powder and was shaken thoroughly. The solution was titrated with 0.1 N perchloric acid solution using 0.5 ml of 0.1% methyl red indicator (end point pink violet). | 229 |
| | | **Ethionamide** | |
| 1 | Ethionamide in pharmaceuticals | A 10 mL aliquot of standard Ethionamide solution containing 1.5-15 mg of Ethionamide was measured accurately and transferred into a 100 mL titration flask, 5 mL of 2M $H_2SO_4$ was added and titrated immediately against 0.01M $KMnO_4$ to a first appearance of pink color. | 98 |
| 2 | Ethionamide in pharmaceuticals | A 10 mL aliquot of the pure Ethionamide solution containing 2-10 mg of drug was placed in a 100 mL titration flask. 25 mL of saturated sodium bicarbonate was added followed by 1 mL starch indicator. The content was titrated with standard iodine solution to a blue end point. | 98 |
| 3 | Ethionamide in pharmaceuticals | A 10 mL aliquot of the drug solution containing 2-9 mg of Ethionamide was measured accurately and transferred into a 100 mL titration flask followed by the addition of 5 mL of 2M HCl. Two drops of methyl orange indicator were added and content titrated vs 5 mM bromate-bromide mixture to a colorless end point. | 98 |

## 8. Other Methods:

## A. Liquid Chromatography/Mass Spectrometry Methods:

**TABLE 12: LIQUID CHROMATOGRAPHY/MASS SPECTROMETRY METHODS**

| S. no. | Drug / Sample | Method | Stationary Phase | Mobile phase | Flow Rate (ml/ min) | Detection/ m/z | Ref. no. |
|---|---|---|---|---|---|---|---|
| | | | **Isoniazid** | | | | |
| 1 | Isoniazid in dog plasma | LC–MS | C18 | 0.1% formic acid:acetonitrile (91:9, v/v) | 1 | Mass spectrometric 138 | 230 |
| 2 | Isoniazid levels in small hair samples | LC/MS-MS | Phenomenex Synergi Polar-RP (100 mm x 2.1 mm, 2.5 µm) | Water with 0.2% (v/v) formic acid | 0.4 | Mass spectrometric 79.0 | 231 |
| 3 | Isoniazid | SFC-MS/MS | Inertsil ODS C18 (150 mm x 4.6 mm, 5 µm) | Dichloromethane: methanol: ethyl acetate: formic acid(70:30:0.5:0.1, v/v/v/v) (15%) and supercritical $CO_2$ (85%) | - | Mass spectrometric138 | 232 |
| | | | **Isoniazid in combination with other drugs** | | | | |
| 4 | Isoniazid (INH) and Ethambutol (EMB) in dried blood spots | LC/MS-MS | Kromasil C18 (150 mm x 4.6 mm, 5 µm) | 0.1% formic acid in water and methanol (35:65, v/v) | 0.8 | Mass spectrometric INH 138.10 →121.10 EMB 205.20 → 116.10 | 233 |
| 5 | Isoniazid (INH) and Ethambutol (EMB) in human plasma | LC/MS-MS | Atlantis Waters C18 (150 mm x 2.1 mm, 3 µm) | Methanol: water: formicacid (10:90:0.3, v/v/v) | 0.20 | Mass spectrometric INH= 205 → 116 EMB= 130 → 60 | 234 |
| | | | **Rifampicin** | | | | |
| 1 | Rifampicin in human plasma | LC/MS-MS | Kinetex C18 (50 mm x 2.1 mm, 2.6 µm) | 0.1% formic acid in water and acetonitrile in gradient elution program | 0.5-0.9 | Mas spectrometric 823.4 → 107.1 and 823.4 → 163.1 | 235 |
| 2 | Rifampicin in human plasma and cerebrospinal fluid | LC/MS-MS | Hypersil–Hypurity C18 (150 mm x 2.1 mm, 5 µm) | ACN containing formic acid (0.05%, v/v) and 15 mMammonium formate buffer (pH 5) in gradient elution program | 0.35 | Mass spectrometric 823.4 → 791.4 | 236 |
| 3 | Rifampicin in plasma | LC/MS-MS | BDS Hypersil Gold C18 (50 mm x 3 mm) | Methanol: 2 mM ammonium acetate (80:20, v/v) | 0.2 | Mass spectrometric | 237 |
| 4 | Rifampicin (RIF) in rat plasma | UPLC-MS/MS | BEH C18 (50 mm x 2.1 mm, 1.7 µm) | Acetonitrile and water (both containing 0.1 % formic acid) in gradient elution program | 0.7 | Mass spectrometric 823.8 | 238 |
| | | | **Ethambutol** | | | | |
| 1 | Ethambutol in human plasma | UFLC-MS | Phenomenex Gemini C18 (50 mm x 2.0 mm, 5 µm) | Acetonitrile: water (pH 2.4 adjusted with 0.5%formic acid) (80:20, v/v) | 1.5 | Mass spectrometric 205 | 239 |
| 2 | Ethambutol in its dosage form and human urine | SFC-MS/MS | Inertsil ODS-C18 (100 mm x 4.6 mm, 5 µm) | Dichloromethane: methanol: formic acid (70:30:0.1, v/v/v) and supercritical $CO_2$ | 0.3 & 2 | Mass spectrometric 205.1 | 240 |
| | | | **Ethambutol in combination with other drugs** | | | | |
| 3 | Ethambutol and Pyrazinamide in human plasma | LC-MS/MS | Chromolith SpeedROD RP-18e (50 mm x4.6 mm, 2 µm) | 0.1%trifluoroacetic acid in water and 0.1% trifluoroacetic acid in methanol in gradient elution program | - | Mass spectrometric | 241 |
| | | | **Pyrazinamide** | | | | |
| 1 | Pyrazinamide in human plasma | LC-MS/MS | Hypersil, Gold (50 mm x 4.6 mm, 5 µm) | Methanol: 0.1 % Formic Acid in 10 mM ammonium | 0.4 | Mass spectrometric 124.100 → 79.160 | 242 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | | formate (90:10, v/v) | | | |
| | | | | **Pyrazinamide in combination with other drugs** | | | |
| 2 | Pyrazinamide & Isoniazid in its dosage | SFC-MS/MS | Inertsil ODS C18 (150 mm x 4.6 mm, 5 µm) | Dichloromethane: methanol: formic acid (50:50:0.1, v/v/v). Supercritical carbon dioxide (SC-$CO_2$) | 0.3 & 2 | Mass spectrometric PYZ= 130 → 60 INH= 160 → 100 | 243 |
| 3 | Pyrazinamide (PYZ), Isoniazid (INH) and Ethambutol (EMB) in serum | LC-MS/MS | Waters C18 analytical (100 mm x 2.0 mm, 3 µm) | Acetonitrile, water and 200 mM ammonium acetate buffer pH 5.0 in gradient elution program | 0.5 | Mass spectrometric PYZ= 81 → 124 INH= 121 → 138.1 ETB = 116.1 → 205.1 | 244 |
| 4 | Pyrazinamide (PYZ), Rifampicin (RIF), Isoniazid (INH), Acetyl Isoniazid (AcINH) & Ethambutol (EMB) in Human Plasma | LC-MS/MS | Gemini C18 (150 mm x 4.6 mm; 4.6 µm) | Methanol: 5 mM ammonium acetate pH 3.5 in gradient elution program | 0.6 | Mass spectrometric PZA: 124 / 81 RIF: 823.46 / 791.49 INH: 138.00 / 121.00 AcINH: 180 / 121 EMB: 205.16 / 116.13 | 245 |
| | | | | **Rifabutin in combination with other drugs** | | | |
| 1 | Rifabutin and 25-O-deacetyl Rifabutin in human plasma | LC-MS/MS | Rp (30 mm x 2.1 mm, 3 µm) | Methanol: water: acetic acid in gradient elution | - | Mass spectrometric For Rifabutin 847.5 → 815.7 For 25-O-Deacetyl Rifabutin 805.7 → 773.7 | 246 |
| 2 | Rifabutin (RBT) & Lopinavir (LPV) in human plasma | LC-MS/MS | HS C18 (50 mm x 4.6 mm, 5 µm) | 85% acetonitrile in ammonium acetate buffer (10mM, pH 4.5) | 0.7 | Mass spectrometric RBT= 847.7 → 815.4 LPV= 629.6 → 447.4 | 247 |
| | | | | **Rifapentine** | | | |
| 1 | Rifapentine in dried blood spot sample | LC-MS/MS | BEH C8 (50 mm x 2.1 mm, 1.7 µm) | 5Mm ammonium formate in water and 3% DMSO in acetonitrile in gradient elution program | - | Mass spectrometric 877.6 → 845.5 | 248 |
| | | | | **Cycloserine** | | | |
| 1 | Cycloserine in blood plasma | HPLC/MS | Acclaim C18 (150 mm x 2.1 mm, 3 µm) | Formic acid (0.1%) and MeCN (55:45, v/v) | 0.3 | Mass spectrometric | 249 |
| 2 | Cycloserine in healthy rat blood and lung tissues | HPLC-MS/MS | C18 (150 mm x 4.6 mm, 5 µm) | Acetonitrile containing 2mM ammonium formate and 0.1% aqueous formic acid (35:65, v/v) | 0.001 | Mass spectrometric | 250 |
| 3 | Cycloserine in 50µL of human plasma | LC-MS/MS | C18 | Acetonitrile & 0.5% formic acid buffer (60:40, v/v) | 0.8 | Mass spectrometric | 251 |
| 4 | Cycloserine in human plasma | LC-MS/MS | Shim-pack XR-ODS (100 mm x2.0 mm, 2.2 µm) | Methanol & 0.01% formic acid (70:30, v/v) | - | Mass spectrometric | 252 |
| | | | | **p-Aminosalicylic Acid** | | | |
| 1 | p-Aminosalicylic acid | LC-MS/MS | Phenomenex Synergi Hydro-RP (150 mm x 2.0 mm, 4µm) | Methanol: 0.2% Formic acid (40:60, v/v) | 0.3 | Mass spectrometric 80.2 → 136.2 | 253 |
| | | | | **Bedaquiline** | | | |
| 1 | Bedaquiline in human serum | LC-MS/MS | HyPURITY C18 (50 mm x 2.1 mm, 3µm) | Purified water, acetonitrile, and an aqueous buffer (containing ammonium acetate [10g/liter], acetic acid [35mg/liter], and | 0.5 | Mass spectrometric 555.1 → 58.4 | 254 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | | trifluoroacetic anhydride [2ml/liter] in water) in gradient elution program | | | |
| 2 | Bedaquiline in human plasma | HPLC-MS/MS | Agilent ZORBAX SB-18 (100 mm x 2.1 mm, 3.5 µm) | Methanol: 5mM ammonium formate(containing 0.1% foemic acid solution) (85:15, v/v) | 0.3 | Mass spectrometric 555.2 → 58.3 | 255 |
| 3 | Bedaquiline in hair | LC-MS/MS | Phenomenex Synergi Polar RP (100 mm x 2.1 mm, 2.5 µm) | Water with 1% formic acid & acetonitrile with 0.4% formic acid in gradient elution program | 0.3 | Mass spectrometric 557.1 → 58.1 | 256 |
| | | | | **Delamanid** | | | |
| 1 | Delamanid in mouse plasma | LC-MS/MS | Capcell Pak C18 MG (50 mm x 2.0 mm, 3 µm) | Purified water-formic acid (1000:2, v/v) and methanol-formic acid (1000:2, v/v) in gradient elution program | 0.2 | Mass spectrometric 535 → 352 | 257 |
| 2 | Delamanid in human plasma | UHPLC-MS/MS | Acquity waters BEH C18 (50 mm x 2.1 mm, 1.7 µm) | A) Ammonium bicarbonate and ammonium hydroxide in water<br>B) Ammonium hydroxide in methanol<br>- Use solution A & B in gradient elution program | 0.5 | Mass spectrometric 535.1 → 352.2 | 258 |
| | | | | **Pretomanid** | | | |
| 1 | Pretomanid (PA-824), Moxifloxacin (MOX) and Pyrazinamide (PYZ) in rat plasma | LC-MS/MS | An Inertsil ODS C18 (150 mm x 4.6 mm, 5 µm) | Methanol & 0.03% triethylamine in water (85:15, v/v) | 0.5 | Mass spectrometricPA-824= 360.1→175.0<br>MOX= 402.1→260.0<br>PYZ= 81.2 | 259 |
| | | | | **1st Line Anti-TB** | | | |
| 1 | Pyrazinamide, Rifampicin, Isoniazid, & Ethambutol | HPLC-MS/MS | C18 | Methanol in 0.3% formic acid and water in gradient elution program | - | Mass spectrometric | 260 |
| 2 | Pyrazinamide, Rifampicin, Isoniazid, & Ethambutol | UPLC-MS/MS | Acquity UPLC HSS T3 (150 mm x 2.1 mm, 1.8 µm) | Water + 0.05% of formic acid and Acetonitrile + 0.05% of formic acid in gradient elution program | - | Mass spectrometric | 261 |
| 3 | Pyrazinamide, Rifampicin, Isoniazid, & Ethambutol in human plasma & PBMCs | UPLC-MS/MS | Waters HSS T3 (150 mm x 2.1 mm, 1.8 µm) | - | - | Mass spectrometric | 262 |
| | | | | **2nd Line Anti-TB** | | | |
| 1 | Nine second-line anti-tuberculosis drugs | UPLC-MS/MS | Waters HSS T3 column (50.0 mm x 2.1 mm, 1.8 µm) | 10 mM ammonium formate in 0.1% formic acid and acetonitrile in 0.1% formic acid in gradient elution program | 0.2 | Mass spectrometric | 263 |

## B. Capillary Electrophoresis:

**TABLE 13: CAPILLARY ELECTROPHORESIS**

| S. no. | Drug /Sample | Description | Ref. No. |
|---|---|---|---|
| | | **Isoniazid** | |
| 1 | Isoniazid (INH) | Capillary electrophoresis method coupled with chemiluminescent (CL) detection was proposed for the analysis of isoniazid based on the enhancement effect of INH to CL emission of luminol-periodate potassium reaction. Under the optimal conditions, INH can be assayed in the range of $7.0 \times 10^{-7}$ to $3.0 \times 10^{-5}$ g/mL (R (2) = 0.9990) with a limit of detection of $3.0 \times 10^{-7}$ | 264 |

| | | | |
|---|---|---|---|
| | | g/mL (signal-to-noise ratio of 3). The whole analysis process can be completed within 2.5 min with a theoretical plate number of 6258. | |
| | | **Ethambutol** | |
| 1 | Ethambutol (EMB) | CE with capacitively coupled contactless conductivity detection. The separation of EMB and its main product of degradation were achieved in less than 3 min with a resolution of 2.0. Using the best separation conditions, linearity of 0.9976 ($R^2$, five data points), the sensitivity of $1.26x10^{-4}$ V min µmol/L, and LOD and quantification of 23.5 and 78.3 µmol/L, respectively, were obtained. | 265 |
| | | **Rifabutin** | |
| 1 | Rifabutin and human serum albumin in pharmaceutical formulations | Capillary zone electrophoresis (CZE) was used for simultaneous determination of rifabutin and human serum albumin. CE conditions: a quartz capillary tube (internal diameter 75mm, effective length 50cm, total length 60cm), the capillary temperature was 25°C, the voltage applied to the capillary tube was +20kV, the UV detection wavelength was 214nm, hydrodynamic injection of the sample was performed at 30mbar for 5s, tetraborate buffer solution (0.01M, pH9.2). The obtained results are characterized by high efficiency (number of theoretical plates up to 260,000) and sufficient sensitivity (LOQ starting from 0.02µg/ml for RFB). | 266 |
| | | **p-Aminosalicylic Acid** | |
| 1 | p-Aminosalicylic acid and its N-acetylated metabolite in human urine | A capillary zone electrophoresis method has been developed for the determination of p-amino salicylic acid (PAS) and its metabolite, N-acetyl-p-aminosalicylic acid (N-acetyl-PAS), in urine. A good separation of the analytes is achieved in a run time of 12 min (15 min total, including capillary wash). A linear relationship was observed between time-normalized peak area and the concentration of the parent and metabolite with correlation coefficients greater than 0.9990. | 267 |

## C. Flow Injection Analysis:

**TABLE 14: FLOW INJECTION ANALYSIS**

| S. no. | Drug /Sample | Description | Ref. No. |
|---|---|---|---|
| | | **Ethambutol** | |
| 1 | Ethambutol in synthetic urine | FIA using a graphite-polyurethane composite electrode as an amperometric detector. In order to characterise the electrochemical behaviour of ethambutol at pH = 8.0 voltammetric studies were performed. The detector was assembled in a flow injection apparatus and operated at +1.2 V (vs. Ag/AgCl (NaCl sat.)). The linear response for the method was extended up to a 1.1 mmol $L^{-1}$ ethambutol solution with a detection limit of 0.0634 mmol $L^{-1}$. The reproducibility of current responses for injections of 0.7 mmol $L^{-1}$ ethambutol solution was evaluated to be 5.1% (n = 30) and the analytical frequency was 161 determinations $h^{-1}$. | 268 |
| | | **p-Aminosalicylic Acid** | |
| 1 | p-Aminosalicylic acid derivatives | FIA with spectrophotometric detection (λ 510 nm). The best conditions were attained using a mixture of ethanol (methanol) and a buffer solution of pH 6.68 (30: 70 vol %). The analytical range for the analytes was 0.08-5.0 µg/ml. | 269 |

## D. Chemiluminescence Method:

**TABLE 15: CHEMILUMINESCENCE METHOD**

| S. no. | Drug /Sample | Description | Ref. No. |
|---|---|---|---|
| | | **Rifampicin** | |
| 1 | Rifampicin | Rifampicin can enhance the chemiluminescence (CL) of peroxomonosulfate-cobalt (II) system, and the CL intensity is strongly dependent on the rifampicin concentrations. Based on this phenomenon, a rapid and sensitive flow injection CL method was developed for the determination of rifampicin. The relative CL intensity was linear with the rifampicin concentration over the range of 5×10 to 1×10 g/mL (r=0.9991), the detection limit was 7×10 g/mL (S/N=3), and the relative standard deviation was 2.7% for 6×10 g/mL rifampicin (n=11). | 270 |

**CONCLUSION:** From all information given in the analytical review, it can be concluded that various UV-Visible spectrophotometric, Spectrofluorimetric, High-performance liquid chromatography (HPLC), High-performance thin layer chromatography (HPTLC), Gas chromatography (GC), Micellar electro-kinetic capillary chromatography, Electrochemical, Titrimetric, Liquid chromatography / Mass spectrometry (LC/MS), Capillary electrophoresis, Flow injection analysis, and Chemiluminescence were used for the determination of the first line, oral second line and newer anti-TB drugs alone and in combination. These methods have been successfully used on a routine basis and allow the quantification of the drugs in raw materials, pharmaceutical formulations, and biological matrices in a short analytical time. These all methods are sensitive, simple, fast, accurate, and

reproducible, as well as possess excellent linearity & precision characteristic. These observations make it possible to anticipate the use of these methods in future analytical research work for Anti-TB drugs.

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